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# Prevalence of *Campylobacter jejuni* in Small-Scale Broiler Operations and the Effects of Sulfadimethoxine Administered to Control *C. jejuni* Infection in Broilers

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PREVALENCE OF *CAMPYLOBACTER JEJUNI* IN SMALL-SCALE BROILER  
OPERATIONS AND THE EFFECTS OF SULFADIMETHOXINE ADMINISTERED  
TO CONTROL *C. JEJUNI* INFECTION IN BROILERS

A Dissertation

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agriculture and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

in

School of Nutrition and Food Sciences

by

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## ABSTRACT

*Campylobacter* spp. particularly *C. jejuni* has been recognized as one of the most prevalent causes of foodborne bacterial illnesses in humans. Most previous studies have focused on the transmission routes of *C. jejuni* from commercial flock farms to the final retail product. To date, no *in vivo* studies have addressed the efficacy of sulfadimethoxine in the control of *C. jejuni* in poultry. This dissertation research proceeds along two lines of investigation. The objectives of the first line of investigation are to determine the enumeration of *Campylobacter* spp. and the prevalence on both *Campylobacter* spp. and *C. jejuni* on live egg shells, to detect the presence and extent of *Campylobacter* spp. and *C. jejuni* in live birds raised in battery-cage and cage-free systems and to determine to what extent these bacteria are present in drinking water, feed, enclosures and troughs. The objectives of the second line of investigation are to determine the effects of sulfadimethoxine antibiotic on the enumeration of *Campylobacter* spp. and the prevalence on both *Campylobacter* spp. and *C. jejuni* in broilers and to examine the effects of sulfadimethoxine antibiotic treatments on the enumeration of *Campylobacter* spp. and prevalence of *Campylobacter* spp. and *C. jejuni* from likely sources of cross-contamination to include drinking water, feed, enclosures and troughs. The results from the first line of investigation suggest that the vertical transmission of these bacteria from egg surfaces to newly hatched chicks is not a significant risk factor. Additionally, this study found that the horizontal transmission of *Campylobacter* spp. and *C. jejuni* among live broilers is significantly higher ( $P < 0.05$ ) raised in the cage-free system than in the battery-cage system. Prevalence of *Campylobacter* spp. and *C. jejuni* in the potential abiotic sources of cross contamination (drinking water, feed, enclosures and troughs) in the cage-free system was significantly higher than in the battery-cage system. The results from the second line of investigation also found that



drinking water may be a prime source of *Campylobacter* spp. and *C. jejuni* cross contamination.

The use of antibiotic sulfadimethoxine can reduce the enumeration and prevalence of *Campylobacter* spp. and the prevalence of *C. jejuni*.

## CHAPTER 1 INTRODUCTION

*Campylobacter jejuni* is an emerging food pathogen with a worldwide distribution. Infections caused by this organism are characterized by self-limiting watery and bloody diarrhea (Altekruse et al., 1999; Friedman et al., 2000; Skirrow and Blaser, 2000). The Center for Disease Control and Prevention (CDC) estimates that approximately 400 million cases of *Campylobacter*-associated gastroenteritis occur annually (Rao et al., 2001; Van et al., 2001) with an economic impact estimated in excess of \$2 billion (Allos, 2011).

Approximately 70% of human illnesses due to *Campylobacter* spp. are caused by the consumption or handling of raw or undercooked poultry (Friedman et al., 2000; Mead et al., 1999). Additionally, *Campylobacter* spp. can be transmitted via contact with infected animals or their feces. Many animals carry *Campylobacter* spp. asymptotically and shed the bacterium in their feces. Poultry, particularly broiler chickens, frequently harbor the bacterium.

Currently, the ecology of *Campylobacter* spp. in the poultry reservoir is poorly understood, particularly with respect to the sources of infection and routes of transmission. It is thought that both vertical and horizontal transmission may affect the immune status of the poultry host (Ridley et al., 2011; Ellis-Iversen et al., 2012).

Intervention strategies for *Campylobacter* infection in poultry should consider the complex nature of its transmission and may require the use of multiple approaches that target different segments of the poultry production system (Irene et al., 2010). Most studies have concentrated on the transmission routes from commercial farm flocks to carcasses after slaughter and to retail products (Dickins et al., 2002; Takahashi et al., 2006; Lienau et al., 2007; Praakle-Amin et al., 2007; Ellerbroek et al., 2010).

Interest in animal welfare issues has spurred an increased interest in free range, small scale, and local poultry production. With these open air, less controlled environments an increased infection rate of *Campylobacter* might be an issue. Therefore, there is a need to control infection rates and identify potential cross contamination vectors associated with small-scale poultry production.

According to the US Food and Drug Administration (2013), sulfadimethoxine is an antibiotic that had been administered in commercial poultry production to treat a variety of infections. These include respiration illnesses, coccidiosis, fowl cholera, and coryza (Wang et al., 2012). Sulfadimethoxine inhibits the bacterial synthesis of folic acid (pteroylglutamic acid) from para-aminobenzoic acid (Vree and Hekster, 1987).

To date, there have been no *in vivo* studies on the efficacy of sulfadimethoxine in the control of *C. jejuni* in broilers in small-scale poultry operations. Although largely unknown, the positive potential effects of sulfadimethoxine antibiotic treatment to control *C. jejuni* infection in chickens are explored.

The present dissertation research is divided into two parts. In the first part, one objective is to determine the prevalence and enumeration of *Campylobacter* on the shells of eggs received from the hatchery during incubation. A second objective is to detect the presence and extent of *Campylobacter* in live birds over a six week period in the battery-cage and the cage-free systems. A third objective is to determine whether and to what extent *Campylobacter* is present in the drinking water, feed, enclosures and troughs of the experimental birds.

In the second part of this dissertation research, one objective is to determine the effects of sulfadimethoxine antibiotic treatments on the enumeration of *Campylobacter* spp. and the prevalence on both *Campylobacter* spp. and *C. jejuni* in live birds over two separate six-week

periods. The second objective is to examine the effects of sulfadimethoxine antibiotic treatments on the enumeration of *Campylobacter* spp. and prevalence of *Campylobacter* spp. and *C. jejuni* from the likely sources of cross-contamination including drinking water, feed, enclosures and troughs.

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## CHAPTER 2 LITERATURE REVIEW

### 2.1 GENERAL CHARACTERISTICS

*Campylobacter* was first recognized as the cause of infectious abortion in sheep during the early 1900s by McFadyean and Stockman (Butzler, 2004). *Campylobacter* species are classified in the family *Campylobacteraceae* (Vandamme, 2000). The generic name *Campylobacter* is derived from the Greek words *campylos*, which means curved and *baktron*, which means *rod*. The name *Campylobacter* or *curved rod*, therefore, describes the appearance of this organism under the microscope (Blaser and Cody, 1986). *Campylobacter* is a gram negative, slender, spiral-curved rod that causes intestinal infectious diseases worldwide (Keener et al., 2004).

The members of the genus *Campylobacter* (*C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, *C. helveticus*, *C. fetus*, *C. hyointestinalis*, *C. mucosalis*, *C. concisus*, *C. curvus*, *C. showae*, *C. rectus*, *C. sputorum*, and *C. gracilis*) are associated with a wide variety of diseases in humans and animals although some are considered commensals (Hald et al., 2000). Within the genus, three species: *C. jejuni*, *C. coli*, and *C. lari* are known as thermophilic members of the genus and of clinical significance as they are the dominant causative agents of human campylobacteriosis (Dekeyser et al., 1972; Jacobs-Reitsma, 2000). *C. jejuni* has been recognized as an important cause of food-borne illness in humans since the late 1970s (Butzler, 2004; Skirrow, 1977). *C. jejuni* grows best in an atmosphere containing approximately 3-8% O<sub>2</sub> and 5-15% CO<sub>2</sub> (Doyle, 1990; Nachamkin, 1999; Rowe and Madden, 2000). Although most *Campylobacter* spp. grow at 37°C, *C. jejuni* and *C. coli* show optimal growth at 42°C (Blaser, 2000; Doyle, 1990; Shane and Montrose, 1985).

## 2.2 *CAMPYLOBACTER* SPP. IN HUMANS

*Campylobacter* spp. is one of the leading causes of bacterial gastroenteritis, causing an estimated 2.1-2.4 million cases in the U.S. annually (Samuel et al., 2004; Altekruze et al., 1999). These infections result in 13,000 hospitalizations, 100 deaths with costs exceeding over \$1 billion annually. The infectious dose varies depending on both host and bacterial factors and ranges from 500-10<sup>6</sup> organisms (Keener et al., 2004; Smith, 1995). Once ingested, the typical incubation period is 24-72 hours. Symptoms include severe acute diarrhea, with variable fever, myalgia, and headache. Infection is typically self-limiting in healthy individuals with resolution of symptoms within 1 week. However, in immunocompromised patients, infection often leads to bacteremia with significant mortality (Coker et al., 2002).

Four species (*C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*) are known as thermophilic *Campylobacters* and are clinically significant due to their dominant causative agents of human campylobacteriosis (Keener et al., 2004; Jacobs-Reitsma, 2000). *C. jejuni* is the predominant species that causes bacterial gastroenteritis in the U.S. and other developed countries, with *C. coli* placing second (Lastovica, 2006). In the U.S., campylobacteriosis and salmonellosis are the leading causes of bacterial foodborne illness. Transmission of *Campylobacter* spp. to humans generally occurs by either ingestion of contaminated food or water or by direct contact with fecal material from infected animals or persons.

In humans, there are two types of illnesses associated with *Campylobacter* spp. infections. These are intestinal and extra-intestinal. Two types of diarrhea are usually observed with campylobacteriosis. One type is an inflammatory diarrhea, with slimy, bloody stools containing leukocytes. The second type is non-inflammatory diarrhea, with watery stools and the absence of blood and leukocytes (Wassenaar, 1997). In some cases, intense abdominal pain,

headache, fever, cramping and vomiting can occur. Serious complications can arise such as Reiter's syndrome, Gullain-Barré syndrome, osteomyelitis, pancreatitis, nephritis, myocarditis, cystitis, septic abortion, and bacteremia (Keener et al., 2004; Winer, 2001; Altekruse et al., 1999). Although campylobacteriosis does not usually lead to death, approximately 700 people in the U.S. succumb to this disease, often due to secondary complications (Saleha et al., 1998). A concern for those suffering from campylobacteriosis is that they might suffer from neurological sequelae months or even years afterwards. Two neuropathies associated with *C. jejuni* infections are Gullain-Barré syndrome (GBS) and Miller Fisher syndrome (MFS). Both of these syndromes are characterized by acute or sub-acute immune mediated neuropathies.

Gullain-Barré syndrome is characterized by alexia, motor paralysis, an acellular increase in the total protein content in the cerebrospinal fluid and an inflammatory demyelinating polyradiculoneuropathy (Winer, 2001; Willison and O'Hanlon, 2000). Gullain-Barré syndrome occurs in approximately 1 out of 100,000 people (Winer, 2001). The prevalence within infected people is estimated to be 1 out of 1,000 and can be even less depending on the strain (Nachamkin, 2002). Gullain-Barré syndrome cases are associated with nerve roots, causing mononuclear infiltration of peripheral nerves and this eventually leads to primary axonal degeneration or demyelination. Molecular mimicry is believed to be the cause of GBS because a few peripheral nerves of the human neurological system share similar molecules with antigens on the surface of *C. jejuni* cells (Nachamkin, 2002; Winer, 2001).

*C. jejuni* contain a lipopolysaccharide structure (LPS) attached to the outer membrane, the core oligosaccharides of its LPS contain ganglioside-like structures which are similar to certain human gangliosides (Ang et al., 2001; Perez and Blaser, 1985; Logan and Trust, 1982). The LPS structure is highly antigenic and upon exposure to *C. jejuni*, the immune system



produces antibodies against the LPS structure as an attempt to fight the infection. Due to the similarity of the core oligosaccharides of the LPS and the gangliosides, after the infection, antibodies attack the gangliosides on the neuromuscular junction contributing to the appearance of neurological symptoms (Godschalk et al., 2004; Ang et al., 2001; Lindsay, 1997).

### **2.3 *CAMPYLOBACTER* SPP. IN POULTRY**

*Campylobacter* spp. have been recovered from rivers, coastal waters, shellfish, and vegetables but are routinely found in sheep, cattle, swine, rodents and poultry (Jacobs-Reitsma, 2000; Kemp et al., 2005). Poultry are the most common hosts for *Campylobacter* spp. and is considered the main source of human illness (Mackiw et al., 2008; Vellinga and Van, 2002). More specifically, approximately 70% of human illnesses due to *Campylobacter* spp. are caused by the consumption or handling of raw or undercooked poultry or poultry products (Friedman et al., 2000; Mead et al., 1999). Increased attention has been given to reducing the level of *Campylobacter* spp. in poultry pre- and post-harvest to reduce the level and incidence of raw product contamination (Friedman et al., 2004; Keener et al., 2004; Allos, 2001). The decimal reduction time for *Campylobacter* spp. varies depending on the type of food product but survival kinetics generally involve a rapid decline in numbers followed by a slower rate of inactivation. This may explain the increased survival rate of *Campylobacter* spp. on poultry carcasses due to the high levels of the organisms in the digestive tract at the time of processing. The potential for survival decreases to a few hours at temperatures of 37°C and increases to a few days at temperatures of 42°C.

Prevalence of *Campylobacter* spp. in food animals can exceed 80% thus challenging processors to employ post-harvest pathogen reduction strategies (Corry and Atabay, 2001; Sahin et al., 2002). The prevalence of *Campylobacter* spp. appears to vary by both species and country

(Newell and Fearnley, 2003). In the United States, the prevalence of *Campylobacter* spp. in commercial broilers ranges from 20-90% (Shane, 2000; Stern et al., 2001). In Canada and South America, the prevalence of *Campylobacter* spp. in commercial broilers ranges from 45-48% and 20-96%, respectively (Aho and Hirn, 1988; Newell and Fearnley, 2003; Shane, 2000). In Europe, the proportion of commercial broiler flocks colonized with *Campylobacter* spp. varies from 2.9% to more than 92% (Bouwknegt et al., 2004; Evans and Sayers, 2000; Hald et al., 2000; Heuer et al., 2001) with the lowest flock prevalence (2.9%) observed in Finland (Perko-Makela et al., 2002). In other regions, the prevalence of *Campylobacter* spp. within commercial broiler flocks ranges from 13.6% to 87% in Africa (Cardinale et al., 2004), 24% to 54% in Asia (Newell and Fearnley, 2003), and approximately 42% in Australia (Saleha et al., 1998).

A significantly high prevalence rate of *Campylobacter* spp. contamination can be found in retail poultry and poultry products and is often directly related to the prevalence rate at the farm (Skovgaard, 2007). The average prevalence rate of infected flocks is 44-59% with a range of 3-100% (Nauta and Havelaar, 2008; Humphrey et al., 2007). The number of contaminated broilers accounts for the high incidence of *Campylobacter* spp. in poultry processing plants and on processed birds (Lindqvist and Lindblad, 2008; Allen et al., 2007). The average *Campylobacter* spp. prevalence rate on chicken at retail is 57% with a range of 23% to 100% (Humphrey et al., 2007).

Among *Campylobacter*-positive flocks, *C. jejuni* was the predominant species in poultry especially in commercial broilers. Many studies on the prevalence of *Campylobacter* spp. in commercial broilers have shown that 85- 98% of commercial broiler flocks were colonized by *C. jejuni* whereas, about 2-11% and 1-5% were colonized by *C. coli* and *C. lari*, respectively (Avrain et al., 2003; Evans and Sayers, 2000; Jorgensen et al., 2002).

## 2.4 SOURCES OF POULTRY FLOCK COLONIZATION

Several risk factors can be linked to colonization and transmission of *Campylobacter* spp. in broiler flocks such as vertical transmission (eggshell samples from hatcheries) and horizontal transmission (age of birds, drinking water, feed samples and equipment swabs: enclosures, troughs) (Hiett et al., 2002; Berndtson et al., 1996a; Jones, 2001; Sahin et al., 2002).

The vertical transmission of *C. jejuni* from hen to chick is a controversial theory that states that the bacterium can infect fertile hatching eggs and at hatch, the chicks carry this organism. For example, studies have shown that *C. jejuni* could be isolated from the outer and inner surface of egg shells laid by naturally infected commercial layers or broiler breeders (Doyle, 1984; Shanker et al., 1986). Investigations on vertical transmission have shown that *C. jejuni* potentially may enter the eggshell (Sahin et al., 2003a).

Doyle (1984) reported that *C. jejuni* can penetrate the outer membranes of refrigerated table eggs but did not find that it penetrated into the egg contents. Neill et al. (1985) demonstrated that *C. jejuni* can penetrate into the egg but they did not find that *C. jejuni* had infected the albumin or yolk. Shanker et al. (1986) reported that from the hen, *C. jejuni* can penetrate into the inner membranes of eggs. Chaudhary et al. (1989) found that *C. jejuni* could penetrate the inner and outer membranes and the egg contents of cracked eggs. From eggs obtained from actively shedding broiler breeders and from a commercial hatchery, Sahin et al. (2003a) detected no *Campylobacter* spp. From commercial breeder eggs, Acevedo (2005) found that 1.6% of the egg's interiors and 3.8% of their surfaces were contaminated by *Campylobacter* spp.

Horizontal transmission is also a potential avenue of flock contamination. A single transmission route in flock contamination has not been identified (Ellis-Iversen et al., 2011;

Ridley et al., 2011). Potential sources of horizontal flock infection include age of birds, used litter, untreated drinking water, domestic pets, wildlife species, house flies, insects, farm equipment, workers and transport vehicles (Kazwala et al., 1990; Van de Giessen et al., 1992; Kapperud et al., 1993; Berndtson et al., 1996a; Jacobs-Reitsma, 1997; Evans and Sayers, 2000). However, none of these suspected sources has been conclusively identified as the source of infection for broiler farms.

The age of the chicken plays a major role in horizontal transmission. The main colonization site of *Campylobacter* spp. in chickens is the mucus layer within the lower intestinal tract overlaying the epithelial cells in the ceca and cloacal crypts (Meinersmann et al., 1991). Colonization in broiler chickens is highest in the mucosal crypts of the caeca, but also occurs in the small intestine (Conlan et al., 2007). *Campylobacter* spp., once colonized within the digestive tract, can be found in levels up to  $10^9$  CFU/g of fecal content (Altekruse et al., 1999). Once a broiler is colonized with *Campylobacter* spp., the organisms are usually present throughout the production cycle. Colonization of these sites in natural environments is not usually observed until 14 to 21 days (Evans and Sayers, 2000). Previous studies found that the commercial broiler flocks under the age of 2 - 3 weeks old are rarely detected (Berndtson et al., 1996b; Engvall et al., 1986; Evans and Sayers, 2000; Jacobs-Reitsma, 1997; Newell and Fearnley, 2003; Sahin et al., 2002; Shane, 2000). Once infected, *Campylobacter* spp. has been found in up to 100% of birds tested in a given flock (Keener et al., 2004). Conlan et al. (2007) reported that chickens less than two weeks of age normally are not colonized due to a “lag phase” derived from maternal antibodies that are prevalent in young chicks. Normally the transmission of *Campylobacter* spp. results from fecal-oral transmission and can often contaminate the entire flock within 5 weeks (Keener et al., 2004; Jacobs-Reitsma et al., 1995). Birds aged 42 days tested 100% positive for

*Campylobacter* spp. whereas, birds aged 56 days showed a 90% infection rate (Northcutt et al., 2003). Berndtson et al. (1996b) found that the rate of positive flocks increased with age from week one to week five.

Environmental sampling of feces is common for *Campylobacter* spp. detection and a number of different techniques exist (Keener et al. 2004). Eifert et al. (2003) compared to sampling techniques (fecal swabs vs. environmental surface “drag” samples) and found that environmental swabs of the litter yielded the highest percentage recovery. Other studies using fecal swabs reported similar results (Stern et al., 2002; Bull et al., 2006).

In commercial poultry settings, water is usually not considered the initial source of contamination of broiler flocks but might play a role in cross-contamination (Lindblom et al., 1986; Pearson et al., 1993). *Campylobacter* spp. can be transmitted by the fecal-oral route to water. Adult chickens consume about 0.05 to 0.16 gallons of water per day depending on temperature (Frame, 2008). Swab samples can be taken to test for the presence of *Campylobacter* in water. Positive samples as high as 88% were reported by Berndtson et al. (1996a). Additionally, they found 90 of 300 pooled swab samples from water lines (30%) tested positive for *Campylobacter*.

Several studies have shown that contamination of water usually occurs after colonization of the flock rather than preceeding it (Engvall et al., 1986; Lindblom et al., 1986; Kazwala et al., 1990). Non-chlorinated water including ground water is frequently used as a source for drinking water on poultry farms and has been implicated as the source of *C. jejuni* (Kapperud et al., 1993; Pearson et al., 1993). The presence of this bacterium in streams, rivers and groundwater might be a sign of recent contamination from livestock or wild birds (Houng et al., 2001).

In general, clean dry litter, enclosures, fresh feed and feed devices are not considered primary sources of *Campylobacter* spp. colonization in broilers (Berndtson et al., 1996a). Although the mode is often unclear, these sources can become cross-contamination vectors (Bull et al., 2006). For example, Johnsen et al. (2006) reported 25% (4/16) feed devices tested positive for *Campylobacter* spp.

Flies, mice, and other pests have also been reported as colonization sources. For example, Stern et al. (2001) found 25% of insects caught outside of poultry houses to be *Campylobacter*-positive. Berndtson et al. (1996b) showed that flies can act as carriers of *C. jejuni* for up to 2 days after infection. Feces from vermin have also been reported as sources of contamination (Newell and Wagenaar, 2000).

Because *Campylobacter* spp. transmission to the poultry reservoir is not always clear, a variety of intervention strategies should be considered in its control. As the nature of transmission is complex, multiple approaches that target different segments of the poultry production system should be considered.

## **2.5 ISOLATION AND IDENTIFICATION**

### **2.5.1 Isolation of *Campylobacter* spp.**

A number of *Campylobacter* spp. selective media have been used. These include blood-free media such as modified charcoal cefoperazone deoxycholate agar (mCCDA), charcoal-based selective medium (CSM), and Karmali agar. Blood-containing media such as Skirrow's, Butzler's, Blaser's, Campy-BAP, Preston agar, and Campy CVA agar are used as well (Bolton et al., 2002; Corry, 2000; Mahon and Manuselis, 2000). Semi-solid blood-free selective motility medium is also used.

These selective media contain combinations of antibiotics to which thermophilic *Campylobacter* spp. are intrinsically resistant but other bacteria particularly enteric microbial flora are susceptible (Corry et al., 1995). Antimicrobial agents that are usually used in *Campylobacter* spp. selective media include a combination of cephalosporins such as cephalothin or cefoperazone and vancomycin or bacitracin. Although both cephalothin and cefoperazone have been used in *Campylobacter* spp. selective media, cefoperazone is preferred because some strains of *C. jejuni* such as *C. jejuni* subsp. *Doylei*, *C. coli*, *C. fetus* and *C. upsaliensis* are inhibited by cephalothin (Corry et al., 1995; Nachamkin et al., 1998).

Because *Campylobacter* spp. are sensitive to oxygen, most selective media usually contain substances that help protect them from the toxic effect of oxygen derivatives (Corry, 2000). The most commonly used substances for neutralizing these toxic oxygen derivatives include whole, lysed, or defibrinated blood; charcoal, a combination of ferrous sulfate, sodium metabisulfite, sodium pyruvate, haemin and haematin (Corry et al., 1995).

In addition, enrichment broths have also been formulated to enhance the recovery rates of thermophilic *Campylobacter* spp. These enrichment broths are useful when low numbers of *Campylobacter* spp. are expected in the sample. The most widely used enrichment broths for *Campylobacter* spp. include Preston, Doyle and Roman, Park and Sanders, Hunt and Radle, and Exeter (Bolton and Robertson, 1982; Corry et al., 1995; Corry, 2000; Doyle and Roman, 1982).

*Campylobacter* spp. is isolated by a direct plating method or a selective enrichment method depending on the organism and the enumeration present in the sample (Sahin et al., 2003b). For example, because feces or intestinal/cecal contents from chickens usually contain high numbers of *Campylobacter* spp. organisms, a selective enrichment method is appropriate.

To successfully isolate *Campylobacter* spp. from samples, selective media and optimal incubation conditions are crucial (Forbes et al., 1998). *Campylobacter* spp. are strictly microaerophilic. The ideal atmospheric environment for optimal recovery of *Campylobacter* spp. is 5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>. In general, most laboratories use 42°C as the primary incubation temperature for *Campylobacter* spp. isolation (Nachamkin, 1999). This temperature is the optimal growth temperature of thermophilic *Campylobacter* including *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*; however, it may not be suitable for the growth of several other *Campylobacter* species (Corry, 2000). In order to isolate thermophilic *Campylobacter* spp., the selective agar plates should be incubated between 37°C and 42°C for 24-48 hours under microaerophilic conditions. If no *Campylobacter* spp. colonies are present on the plates, the incubation should be extended up to 96 hours before being reported as negative (Forbes et al., 1998; Nachamkin, 1999).

### **2.5.2 Identification of *Campylobacter* spp.**

*Campylobacter* spp. colonies appear differently depending on the media used. For example, colonies might appear gray to pinkish gray, non-hemolytic, flat and slightly mucoid (Forbes et al., 1998; Kaplan and Weissfeld, 1994; Nachamkin, 1999). When *Campylobacter* spp. are sub-cultured onto freshly prepared moist media, colonies appear along the streak line or swarm on the agar plate. As the moisture content decreases, *Campylobacter* colonies may become round, convex, and glistening with little spreading (Nachamkin, 1999; Shane and Montrose, 1985). Colony and microscopic morphology also are useful to identification of *Campylobacter* spp. (Rowe and Madden, 2000).

*Campylobacter* spp. are gram-negative, curved-rod shaped bacteria that are approximately 0.2 to 0.5 µm wide and about 0.5 to 5 µm long (Mahon and Manuselis, 2000;



Rowe and Madden, 2000). Because *Campylobacter* spp. are not easily visualized with safranin counterstain, carbolfuchsin is used.

Other phenotypic tests, especially oxidase and catalase tests, are used to confirm the identification of some *Campylobacter* spp. (Kaplan and Weissfeld, 1994, Sahin et al., 2003b). Other biochemical tests include hippurate hydrolysis, hydrogen sulfide (H<sub>2</sub>S) production in triple sugar iron agar butts, nitrate reduction, urease production, indoxyl acetate hydrolysis, cephalothin sensitivity, and nalidixic acid susceptibility (Forbes et al., 1998; Sahin et al., 2003b).

Hydrolysis of sodium hippurate is the primary biochemical test used to differentiate *C. jejuni* from other thermophilic *Campylobacter* spp. This is because only *C. jejuni* can hydrolyze sodium hippurate (Blaser, 2000; Sahin et al., 2003b).

Newer techniques have been developed for rapid detection and identification of *Campylobacter* species. Techniques based on antigen-antibody interactions include latex agglutination tests, enzyme immunoassays (EIA), enzyme-linked immunosorbent assay (ELISA), immunoblotting, colony blotting, colony lift immunoassay (CLI) and immunomagnetic separation (IMS). Techniques based on deoxyribonucleic acid (DNA) detection include DNA hybridization assays and polymerase chain reaction (PCR) (Sahin et al., 2003b).

### **2.5.3 Latex Agglutination Immunoassay**

As state previously, latex agglutination tests are used for rapid identification and confirmation of *Campylobacter* spp. (Hazeleger and Beumer, 2000; Mahon and Manuselis, 2000). The tests identify *Campylobacter* spp. at the generic level but not the species (Hazeleger and Beumer, 2000; Kaplan and Weissfeld, 1994; Mahon and Manuselis, 2000; Nachamkin, 1999).

Latex agglutination tests are usually performed after the primary isolation of thermophilic *Campylobacter* spp. They are not intended for direct detection of *Campylobacter* spp. from field samples (Hazeleger and Beumer, 2000; Mahon and Manuselis, 2000; Sahin et al., 2003b). Examples of the commercially available latex agglutination test kits include Campyslide (BBL Microbiology Systems, Cockeysville, MD), Meritec Campy (Meridian Diagnostics, Cincinnati, OH), ID Campy (Integrated Diagnostics, Baltimore, MD), INDX-Campy (PanBio INDX, Inc., Baltimore, MD), and Microscreen *Campylobacter* (Mercia Diagnostics, Guildford, UK) (Hazeleger and Beumer, 2000). Most of these test kits can detect *C. jejuni*, *C. coli*, and *C. lari* (Mahon and Manuselis, 2000). Some of these tests also can detect *C. fetus* and *C. upsaliensis*. Instructions on the use of this test have been published by Hazeleger and Beumer (2000).

## **2.6 ANTIMICROBIAL USE IN POULTRY PRODUCTION**

Antimicrobials in poultry production are used to enhance growth, control natural spoilage processes and prevent or control the growth of pathogenic microorganisms (Tanner, 2000; Tajkarimi et al., 2010). Generally, poultry carry *C. jejuni* asymptomatically and shed the bacterium in their feces. Several studies have addressed the use and efficacy of antibiotics on an array of poultry infections including *Campylobacter* spp. and *C. jejuni*. For the most part, the results are mixed. For example, the *in vivo* study by Carvalho et al (2010) found that a three-phage lytic cocktail administered to chickens resulted in a 2 log CFU/g reduction in *C. jejuni*. In another *in vivo* study using turkeys, Scupham et al. (2010) found that the administration of enrofloxacin, neomycin and vancomycin resulted in a respective decrease of 1, 2 and 4 log CFU/g in *C. jejuni*. Robyn et al. (2013) attempted to inhibit *C. jejuni* in chickens through the administration of live bacterium *Enterococcus faecalis*. As they reported, this bacterium failed to inhibit the growth of *C. jejuni*.

Although there are multiple levels at which *Campylobacter* spp. contamination can be targeted, on-farm control has the greatest impact because the living poultry intestine is the primary amplification point throughout the food chain (Wagenaar et al., 2006; Wagenaar et al., 2008).

### **2.6.1 Use of Sulfonamides**

The general term *sulfonamide* refers to any derivative of sulfanilamide. Sulfonamides are structural analogues of para-aminobenzoic acid (PABA) and compete with PABA for the enzyme dihydropteroate synthetase (DHPS). This action prevents PABA from becoming incorporated into folic acid, a necessary cellular component. Resistance to sulfonamide in Gram-negative bacteria is normally due to the acquisition of drug-resistant variant of DHPS. This resistance has been shown to be transferred horizontally among isolates (Radstrom et al., 1991). In Gram-positive bacteria the most common mechanisms are mutations in the gene encoding DHPS (Swedberg et al., 1998).

Sulfonamides have been used to treat a wide range of infectious diseases in humans and animals including pneumonia, meningitis, malaria, and urinary tract infections. Their broad spectrum of activity rivals that of the tetracyclines. They are used alone or in combination with trimethoprim. Trimethoprim inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF) by binding to bacterial dihydrofolic acid reductase (DHFR) (Roland et al., 1979). Sulfonamides decrease the *de novo* synthesis of DHF while trimethoprim decreases the conversion of new and recycled DHF to THF. Combination of the two drugs results in a synergistic sequential blockade (Swedberg et al., 1979).

In humans, adverse reactions to sulfonamides are relatively common and can occur from direct toxicity or hypersensitivity (Stowe, 1965). Although not a prominent effect, sulfonamides

can produce disturbances in gastrointestinal flora due to their broad spectrum of activity. The most common symptoms are anorexia, nausea and vomiting which occurs in 1% to 2% of patients. Disturbances of the urinary tract, including renal crystalluria, can occur while taking sulfonamides if fluid intake is low. Sulfonamides can cause hypothyroidism by impairing thyroglobulin iodination and coupling of tyrosinases. Hypersensitivity reactions including fever, itching, and skin rashes can occur in up to 3% of patients (Bevill and Huber, 1977).

In poultry, sulfonamides were first used to treat upper respiratory (Delaplane, 1945) and coccidial infections caused by *Eimeria tenella* and *Eimeria necatrix* (Grumbles and Delaplane, 1948, Waletzky and Hughes, 1946). The commonly used sulfonamide in poultry production is sulfadimethoxine and therefore it appropriate for in vivo testing.

### **2.6.2 Effects of Sulfadimethoxine on *C. jejuni***

According to US Food and Drug Administration (2013), sulfadimethoxine is an antibiotic that has been administered in commercial poultry production to treat a variety of infections. These include the respiration illnesses, coccidiosis, fowl cholera, and coryza (Vree and Hekster, 1987; Wang et al., 2012).

To date, there have been no in *vivo* studies on the efficacy of sulfadimethoxine in the control of *C. jejuni* in broilers. Although largely unknown, the positive potential effects of sulfadimethoxine antibiotic treatment to control *C. jejuni* infection in chickens is worthy of exploration.

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## **CHAPTER 3**

### **PREVALENCE AND DISTRIBUTION OF *CAMPYLOBACTER JEJUNI* IN SMALL-SCALE BROILER OPERATIONS**

#### **3.1 INTRODUCTION**

*Campylobacter jejuni* is a common foodborne bacterial pathogen of humans in the United States and other developed countries. The infection caused by this organism is characterized by self-limiting watery and bloody diarrhea (Altekruse et al., 1999; Friedman et al., 2000; Skirrow and Blaser, 2000). The majority of human *Campylobacter* infections result from consumption of undercooked chicken or food contaminated by raw chicken (Stern, 1992; Altekruse et al., 1999; Friedman et al., 2000). In 2013, FoodNet identified 19,056 laboratory-confirmed cases of human *Campylobacter* infection. The number of infections and incidence per 100,000 populations by *Campylobacter* are 6,621 and 13.82 respectively. Of these infected populations, 1,010 people (15%) were hospitalized and 12 people (0.2%) died from food contaminated by *Campylobacter* (FoodNet, 2013). The estimated incidence of infections caused by *Campylobacter* was 14% higher in 2012 compared with 2006-2008 (FoodNet, 2012). An estimated 2.1–2.5 million cases of human campylobacteriosis occur annually in the USA (Blaser, 1997; Altekruse et al., 1999; Friedman et al., 2000).

*Campylobacter jejuni* infects a variety of wild and domestic animals and birds. Commercial poultry is considered the major reservoir of human *Campylobacter* infections (Friedman et al., 2000). Therefore, reduction of *C. jejuni* levels in the poultry production system is essential to public health. In order to reduce or eliminate *Campylobacter* spp. from poultry, it is necessary to understand the ecological aspects of the infection in the reservoir. On-farm production practices can affect pathogen loads on poultry entering slaughter facilities, resulting in cross contamination post-harvest. For the past several decades, a large number of farm-based

studies have been performed to determine the epidemiological features of *C. jejuni* infections (Shane, 1992; Stern, 1992; Friedman et al., 2000; Newell and Wagenaar, 2000).

*Campylobacter jejuni* is highly prevalent in chicken flocks, especially in chickens more than 3 weeks old. The organism is carried in poultry intestinal contents in high amounts, leading to fecal contamination of chicken carcasses in processing plants (Shane, 1992; Stern, 1992; Newell and Wagenaar, 2000). Despite this high colonization rate, infected chickens show little or no clinical signs of illness (Shane, 1992; Stern, 1992). Sources of infection and modes of transmission for *C. jejuni* infection in poultry farms are not well understood. Many studies suggest that horizontal transmission from environmental sources is the major mode of chicken flock infection by *C. jejuni* (Clark and Bueschkens, 1988; Stern, 1992; Pearson et al., 1993; Jacobs-Reitsma et al., 1995; Newell and Wagenaar, 2000). However, several findings suggest that vertical transmission might also play a role in introducing *C. jejuni* from breeders into broiler flocks (Doyle, 1984; Shane et al., 1986; Pearson et al., 1993; Chuma et al., 1997). The complexity of *Campylobacter* transmission and the extensive nature of the colonization might undermine the effectiveness of management-based intervention measures. This problem highlights the need for alternative strategies, such as vaccination, to control *C. jejuni* infection in the poultry reservoir and consequently reduce the risk of human campylobacteriosis (Sahin et al., 2002).

Currently, the ecology of *Campylobacter* spp. in the poultry reservoir is poorly understood, particularly with respect to the sources of infection and routes of transmission. It is thought that both vertical and horizontal transmission may affect the immune status of the poultry host and the environmental conditions in the production system (Ridley et al., 2011; Ellis-Iversen et al., 2012). Intervention strategies for *Campylobacter* spp. infection in poultry



should consider the complex nature of its transmission and may require the use of multiple approaches that target different segments of the poultry production system (Irene et al., 2010). However, most studies have concentrated on the transmission routes from commercial farm flock to carcasses after slaughter and retail products (Dickins et al., 2002; Takahashi et al., 2006; Lienau et al., 2007; Praakle-Amin et al., 2007; Ellerbroek et al., 2010). Interest in animal welfare issues has spurred an increased interest in free range, small scale, and local poultry production. With these open air, less controlled environments an increased infection rate of *Campylobacter* spp. might be an issue. Therefore, there is a need to control infection rates and identify potential cross contamination vectors associated with small-scale poultry production.

The objectives of these experiments are severalfold. One objective is to determine the enumeration of *Campylobacter* spp. and the prevalence on both *Campylobacter* spp. and *C. jejuni* on the shells of eggs received from the hatchery during incubation. A second objective is to detect the presence and extent of *Campylobacter* spp. and *C. jejuni* in live birds raised in the battery-cage and the cage-free systems over a six week period. A third objective is to determine whether and to what extent *Campylobacter* spp. and *C. jejuni* are present in the drinking water, feed, enclosures and troughs of the experimental birds. This research effort might help improve the development of risk management strategies in the industry. Ultimately, these findings might help reduce the risks associated with campylobacteriosis to the consumer.

## **3.2 MATERIALS AND METHODS**

### **3.2.1 Sample Collection**

#### **3.2.1.1 Developing Eggs**

Ninety six incubating eggs housed at the McNeese State University Agricultural Sciences laboratory in Lake Charles, Louisiana were evaluated for *C. jejuni*. Each week, seventy five

eggshells were randomly swabbed using a Sterile Rayon Tipped swab. The sample swabs were placed in individual tubes containing 3 ml of sterile tryptone soya broth (TSB) for enumeration and isolation of *Campylobacter* spp. for 3 weeks until hatching.

#### 3.2.1.2 Broiler Production

Two replications each using 150 broiler chickens (Ross × Ross) obtained from the McNeese State University Research Farm in Lake Charles, Louisiana were conducted. Birds were raised under two different production systems: the Petersime<sup>®</sup> battery-cage system (32°C) with raised wire flooring and the cage-free system with covered pen and wood shavings. The Petersime<sup>®</sup> battery-cage system was divided into 12 pens of equal size (29.4" x 39" x 9.5"). Each pen housed six or seven birds. Individual water and feed troughs were provided for each pen. Feed was procured from the Texas Farm Products Company. This feed contains 18% protein chick grower crumbles and no antibiotics. For each replication, 75 broilers were placed in the cage-free system (150.5" x 202" x 100"). In the cage-free system, drinking water and feed were provided by a single waterer and feeder. Heat lamps were suspended 0.4 m above the litter for temperature regulation. For the battery-cage and the cage-free systems, drinking water and feed were supplied free-choice.

Each week, individual chickens from the battery-cage and the cage-free systems were randomly sampled using Sterile Rayon Tipped Swabs. The sample swabs were placed in a tube containing 3 ml of sterile tryptone broth (TSB) for further analysis.

#### 3.2.1.3 Environment Samples

One hundred forty four samples were randomly collected from drinking water, feed and equipment (enclosures and troughs) for two replications from September, 2013 to February, 2014. Approximately 3-5 g per feed sample and 5-10 ml of drinking water per sample were

collected from the feed troughs and water troughs and placed in sterile bottles. Surface areas of enclosures and troughs were swabbed and immediately placed into tubes containing 3 ml of TSB.

Chicken feces and environment samples were collected using aseptic techniques in sterile containers and were transported to the lab and analyzed at week 1, 2, 3, 4, 5 and 6. Quantitative concentration of *Campylobacter* spp. was determined using the method described by Corry et al. (2003). Isolation of *Campylobacter* spp. in the samples was done using Latex Agglutination tests (Moore et al., 2005; Miller et al., 2008).

### **3.2.2 Bacterial Isolation and Identification**

Immediately upon arrival in the laboratory, the swab samples were whirl-mixed in a shaker incubator (New Brunswick Scientific Excella™ E24/E24R Temperature-Controlled Benchtop Shaker) for approximately 1 h at 37°C and then mixed with a vortexer for 2 min to release the bacteria. Each 0.1 ml of swab sample was aseptically transferred and directly streaked onto modified charcoal cefoperazone deoxycholate agar (mCDDA). The inoculated plates were then incubated at 42°C for 48 h under a microaerophilic environment (5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>). Verification of *Campylobacter* spp. isolated from the sample was done by Latex agglutination tests with a Microgen M46 *Campylobacter* Assay Kit (Microgen Bioproducts Ltd., Camberley, Surrey, United Kingdom). In addition, a hippurate hydrolysis test was performed to confirm *C. jejuni* (Hwang and Ederer, 1975).

### **3.2.3 *Campylobacter* Latex Agglutination Kit**

The Microgen Kit is composed of F46a (Test Latex Reagent): latex particles coated with rabbit antibodies to *Campylobacter* antigens, F46b (Control Latex Reagent): latex particles coated with non-specific rabbit immunoglobulins, F46c (Positive Control): suspension of

inactivated *Campylobacter* antigens reactive with test latex reagent and non-reactive with control latex reagent, and F40 (0.85% Isotonic Saline).

All reagents were allowed to reach room temperature and gently shaken to ensure a homogeneous suspension. One drop (50 µL) of isotonic saline (F40) was dispensed to each of the two ovals of the agglutination slide. An inoculating loop was used to remove several colonies with *Campylobacter*-like morphology. Bacteria were mixed into each of the two drops of isotonic saline (F40) on the slide to form an even suspension. One drop (50 µL) of Control Latex reagent (F46b) was added to one of the bacterial suspensions on the slide. One drop (50 µL) of Test Latex Reagent (F46a) was similarly dispensed to the other bacterial suspension. The bacterial suspensions were mixed with latex reagents using a mixing stick with the Control Latex Reagent (F46b). The mixtures were spread to the edges of the oval areas. The slide was gently rocked to keep the fluid suspensions in constant movement for 2 min to produce agglutination. Strength of the reaction is variable and was assessed according to the following: + reaction: fine, but readily discernible granularity against a milky background, ++ reaction: coarse granularity against a milky background, and +++ reaction: heavy clumping of particles around the periphery of the test oval, against a clear background.

### **3.2.4 Hippurate Hydrolysis Test**

This test is used to determine whether a microorganism, by action of the enzyme hippurate hydrolase, can hydrolyze sodium hippurate to benzoic acid and glycine. The glycine end product is detected by the addition of ninhydrin reagent and through a complex reaction forms a deep purple color that can be easily visualized for the identification of *C. jejuni*.

A 1% aqueous solution of sodium hippurate was prepared and dispensed in 0.4 ml amounts into 0.2 ml centrifuged tubes. Inoculum from 24 h of *Campylobacter* growth of the test

organism was emulsified into a tube of the hippurate solution and incubated in a 37°C for 2 h. After incubation, 0.2 ml of the ninhydrin solution was carefully added down the side of the tube to form an overlay over the cell mixture so not to mix the solutions. Samples were incubated for 10 minutes and observed. A deep purple-blue result indicated a positive test for *C. jejuni*. A colorless result indicated a negative result for the presence of *C. jejuni*.

### **3.2.5 Statistical Analysis**

Statistical procedures were performed using SAS Windows (SAS Institute, 2003). A block design with two replications was randomly assigned to two environmental conditions of chicken production. All calculations were performed with Proc GLM procedures (SAS, 2003) using P=0.05 for significance of Least Squares Means and simple correlation coefficients among variables with a model of chicken production environmental condition and period time testing. When treatment difference is detected, specific comparisons between treatment means at that time point were made with the PDIFF option of LSMEANS.

## **3.3 RESULTS AND DISCUSSION**

### **3.3.1 Egg Samples**

Seventy five egg surface swabs were tested for *Campylobacter* spp. and *C. jejuni* during the three week incubation period. No *Campylobacter* spp. and *C. jejuni* growth was detected (0/75 tests). These results are consistent with those of Sahin et al. (2003) who reported no *Campylobacter* spp. in samples of 500 eggs obtained from actively shedding broilers or of 1,000 eggs obtained from a commercial hatchery. It has been proposed that the vertical transmission of *Campylobacter* spp. from eggs to broilers might be due to abiotic factors. These results suggest that the possibility of vertical transmission of *Campylobacter* spp. and *C. jejuni* specifically from

the surface of eggs to newly hatched chicks is not a significant risk factor in small commercial settings.

### **3.3.2 Environment Samples**

#### **3.3.2.1 Drinking Water**

An important potential source of outbreaks of *Campylobacter* gastroenteritis is contaminated water. The presence of *Campylobacter* spp. in drinking water stations has been reported in other studies. For example, Berndtson et al. (1996) found the presence of *Campylobacter* spp. in broiler house drinking water samples as high as 88%.

This study tested for the presence of *Campylobacter* spp. in drinking water from the battery-cage and the cage-free systems. *Campylobacter* spp. counts in drinking water from the battery-cage system ranged from 0 to 2.10 log CFU/ml and from the cage-free system ranged from 0 to 3.05 log CFU/ml (Figure 3.1). The counts of *Campylobacter* spp. in drinking water stations steadily increased from week 1 to week 6 from the battery-cage and the cage-free systems (Figure 3.1).

The prevalence of *Campylobacter* spp. in drinking water ranged from 0% (0/144 samples) to 66.67% (96/144 samples) in the battery-cage system and ranged from 0% (0/144 samples) to 76.39% (110/144 samples) in the cage-free system (Table 3.1).

Additionally, the specific prevalence of *C. jejuni* through hippurate hydrolysis in drinking water samples ranged from 0% (0/144 samples) to 20.14% (29/144 samples) in the battery-cage system and from 0% (0/144 samples) to 76.39% (110/144 samples) in the cage-free system (Table 3.1). The results of this study clearly show that the prevalence of *C. jejuni* in drinking water samples was significantly higher ( $P < 0.05$ ) in the cage-free system (76.39%; 110/144 samples) than in the battery-cage system (5.55%; 8/144 samples) in week 4 (Table 3.1).

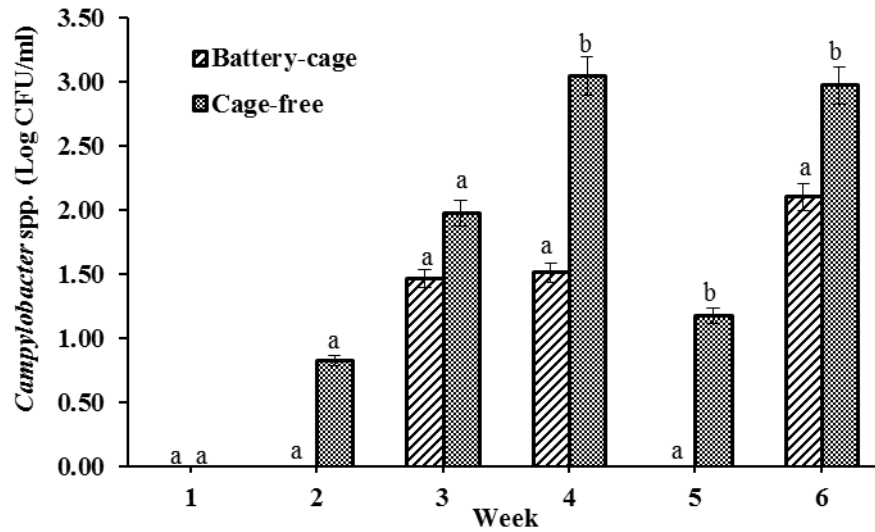


Figure 3.1 *Campylobacter* spp. bacterial counts in drinking water samples from the battery-cage and the cage-free systems from weeks 1 through 6. Data are means from two replications. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 1.7784.

Table 3.1 The prevalence of *Campylobacter* spp. and *C. jejuni* in drinking water samples from the battery-cage and the cage-free systems from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 144)		No. (%) of positive samples of <i>C. jejuni</i> (n = 144)	
	Battery-cage	Cage-free	Battery-cage	Cage-free
1	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
2	ND <sup>a</sup>	96 (66.67) <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>
3	96 (66.67) <sup>a</sup>	96 (66.67) <sup>a</sup>	24 (16.67) <sup>a</sup>	72 (50) <sup>b</sup>
4	68 (47.22) <sup>a</sup>	110 (76.39) <sup>b</sup>	8 (5.55) <sup>a</sup>	110 (76.39) <sup>b</sup>
5	ND <sup>a</sup>	64 (44.44) <sup>b</sup>	ND <sup>a</sup>	64 (44.44) <sup>b</sup>
6	58 (40.28) <sup>a</sup>	44 (30.56) <sup>a</sup>	29 (20.14) <sup>a</sup>	43 (29.86) <sup>a</sup>

ND = Non detectable. Data are means from two replications. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM for *Campylobacter* spp. = 0.0747; SEM for *C. jejuni* = 0.0680.

Drinking water supplied to chickens in the cage-free system is through a single communal system. This type of system lies in close proximity to the soil. Therefore, drinking water in this system can easily become contaminated by chicken feces and other pollutants. Under these conditions, sediments and biofilms may form that serve as an environmental reservoir for *Campylobacter* spp. and *C. jejuni*. Cross contamination from this environment to broilers is a distinct possibility. As noted by Pearson et al. (1993) and Lindblom et al. (1986) drinking water is usually not the primary source of *Campylobacter* spp. and *C. jejuni* contamination but might subsequently contribute to cross-contamination.

These findings suggest that the drinking water used in chicken production, especially in the cage-free system, should be clean and free of chicken feces that can help sustain the growth of *Campylobacter* spp. and *C. jejuni*. Routine monitoring of the drinking watering system must occur frequently. Having fewer birds per drinking water trough may reduce the enumeration of *Campylobacter* spp. and the prevalence of *Campylobacter* spp. and *C. jejuni*.

#### 3.3.2.2 Feed

Due to its low moisture content, fresh feed is an unlikely primary source of *Campylobacter* (Berndtson et al., 1996). However, feed subsequently can be contaminated from other sources such as feces (Shreeve et al., 2000) and can play a role in the transmission through cross-contamination of *C. jejuni* to broilers. In this study, fresh feed from each bag was tested for *Campylobacter* spp. and *C. jejuni* before it was fed to the broilers. Neither *Campylobacter* spp. nor *C. jejuni* was detected. Subsequently, *Campylobacter* spp. counts from feed samples during the six-week period of this study ranged from 0-2.10 log CFU/ml in the battery-cage system and from 0-2.17 logs CFU/ml in the cage-free system (Figure 3.2).



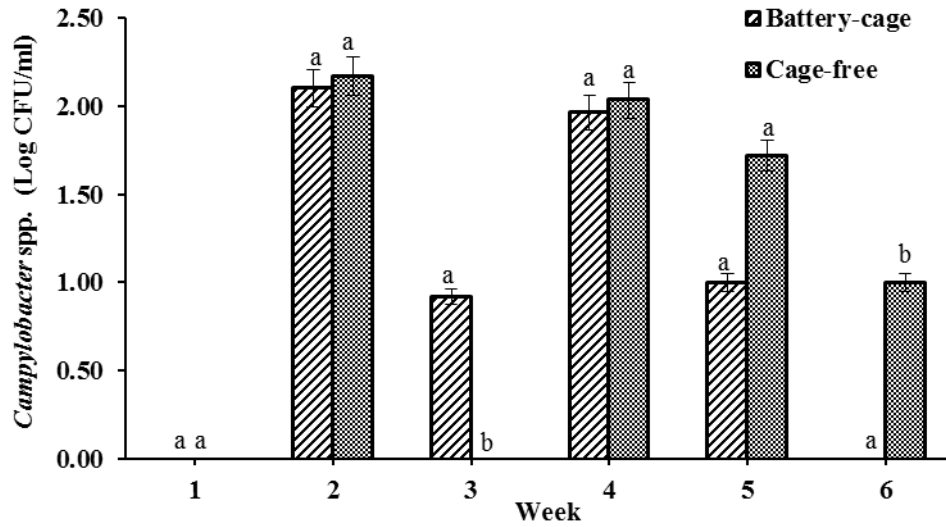


Figure 3.2 *Campylobacter* spp. bacterial counts in feed samples from the battery-cage and the cage-free systems from weeks 1 through 6. Data are means from two replications.

<sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 1.6439.

The prevalence of *Campylobacter* spp. from feed samples in the battery-cage system ranged from 0% (0|144 samples) to 83.33% (120|144 samples) and 0% (0|144 samples) to 35.42% (51|144 samples) in the cage-free system (Table 3.2).

Additionally, the prevalence of the specific bacterium, *C. jejuni*, ranged from 0% (0|144 samples) to 33.33% (48|144 samples) from feed samples in the battery-cage system and from 0% (0|144 samples) to 35.42% (51|144 samples) in the cage-free system (Table 3.2). The results of this study suggest that feed had become cross-contaminated by *Campylobacter* spp. and *C. jejuni*. The most likely sources for this contamination are the route from feces to beak to feed.

Table 3.2 The prevalence of *Campylobacter* spp. and *C. jejuni* in feed samples from the battery-cage and the cage-free systems from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 144)		No. (%) of positive samples of <i>C. jejuni</i> (n = 144)	
	Battery-cage	Cage-free	Battery-cage	Cage-free
1	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
2	120 (83.33) <sup>a</sup>	48 (33.33) <sup>b</sup>	48 (33.33) <sup>a</sup>	ND <sup>b</sup>
3	60 (41.67) <sup>a</sup>	ND <sup>b</sup>	36 (25.00) <sup>a</sup>	ND <sup>b</sup>
4	93 (64.58) <sup>a</sup>	51 (35.42) <sup>b</sup>	34 (23.61) <sup>a</sup>	51 (35.42) <sup>a</sup>
5	16 (11.11) <sup>a</sup>	16 (11.11) <sup>a</sup>	16 (11.11) <sup>a</sup>	16 (11.11) <sup>a</sup>
6	ND <sup>a</sup>	43 (29.86) <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>

ND = Non detectable. Data are means from two replications. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM for *Campylobacter* spp. = 0.0781; SEM for *C. jejuni* = 0.0573.

As with the water supply, it is imperative to maintain clean feed when producing broilers in order to prevent cross-contamination. To that end, fresh dry feed, stored in closed containers, should be used. Feed bins should be periodically disinfected with a 10% chlorine bleach solution (Lacy and Czarick, 1992). Additionally, feed should not be supplied in overabundance so that it becomes fallow.

### 3.3.2.3 Enclosures

In this study, enclosures were randomly sampled for the presence of *Campylobacter* spp. and *C. jejuni*. Because the bacterium cannot survive on dry surfaces, enclosures typically are not considered primary sources of *Campylobacter* colonization. However, enclosures can become contaminated from feces of vermin (Newell and Wagenaar, 2000). A total of 144 enclosure samples for two replications were swabbed from the battery cage and the cage-free systems.

*Campylobacter* spp. counts were detected on the surface of enclosures in both the battery-cage and the cage-free systems. During the six-week period of testing, *Campylobacter* spp. counts from enclosure samples ranged from 0-1.30 log CFU/ml in the battery-cage system and from 0-2.34 log CFU/ml in the cage-free system (Figure 3.3).

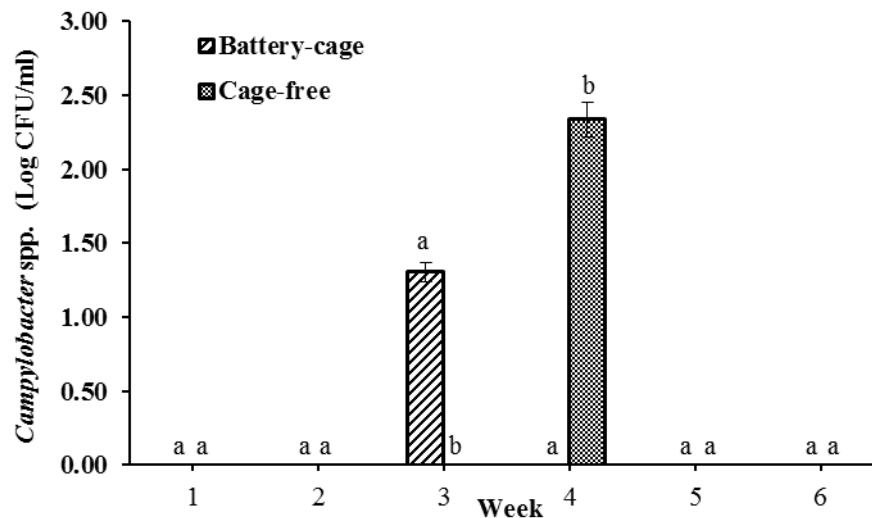


Figure 3.3 *Campylobacter* spp. bacterial counts from enclosure samples from the battery- cage and the cage-free systems from weeks 1 through 6. Data are means from two replications.

<sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 1.3931.

In this study, the prevalence of *Campylobacter* spp. was not found in weeks 1, 2, 5, and 6 in the battery-cage and the cage-free systems (Table 3.3). However, the prevalence of *Campylobacter* spp. was found during the third week at 16.67% (24/144 samples) in the battery-cage system and during the fourth week at 58.33% (84/144 samples) in the cage-free system (Table 3.3). Additionally, through hippulate hydrolysis, the prevalence of specific bacterium *C. jejuni* was not found in the battery-cage system. However, *C. jejuni* was found in the cage-free system at 16.67% (24/144 samples) (Table 3.3).

Table 3.3 The prevalence of *Campylobacter* spp. and *C. jejuni* from enclosure samples from the battery-cage and the cage-free systems from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 144)		No. (%) of positive samples of <i>C. jejuni</i> (n = 144)	
	Battery-cage	Cage-free	Battery-cage	Cage-free
1	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
2	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
3	24 (16.67) <sup>a</sup>	ND <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>
4	ND <sup>a</sup>	84 (58.33) <sup>b</sup>	ND <sup>a</sup>	24 (16.67) <sup>b</sup>
5	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
6	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>

ND = Non detectable. Data are means from two replications. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different, P<0.05. SEM for *Campylobacter* spp. = 0.0557; SEM for *C. jejuni* = 0.0324.

It is uncertain as to why the enclosures in each setting showed evidence of *Campylobacter* contamination for only one week during the six weeks of testing. Although this may appear to be an anomaly, the presence of *Campylobacter* on enclosures, even for a short period of time can have major consequences. That is, cross-contamination, as with drinking water and feed, can occur rapidly through this vector. Therefore, regular sanitation practices designed to clean and disinfect cages should be employed. Specifically, the application of Nolvasan Solution, which contains chlorhexidine diacetate, is the standard for routine cleaning and disinfecting in small-scale poultry operations.

#### 3.3.2.4 Troughs

One hundred forty four trough samples were swabbed from the battery-cage and the cage-free systems for two replications. *Campylobacter* spp. counts in this study ranged from

0-1.55 log CFU/ml in the battery-cage system and from 0-2.26 log CFU/ml in the cage-free system (Figure 3.4).

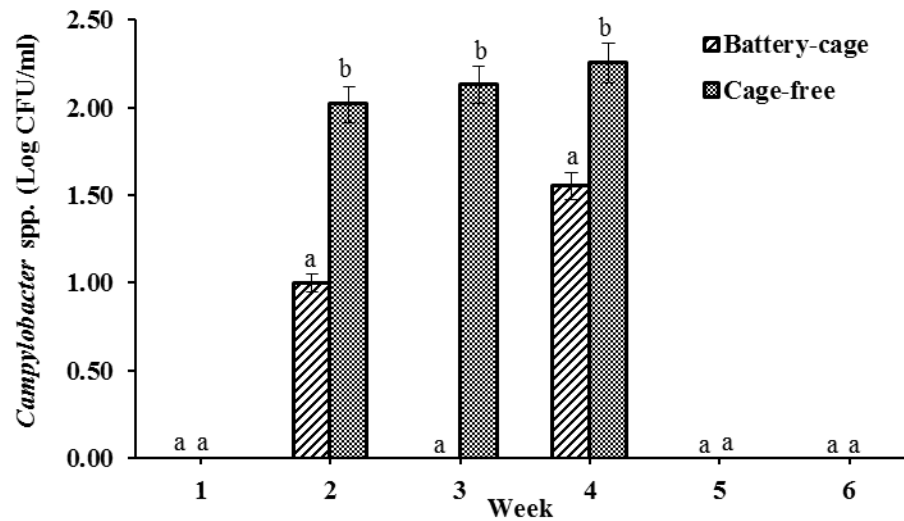


Figure 3.4 *Campylobacter* spp. bacterial counts from trough samples from the battery-cage and the cage-free systems from weeks 1 through 6. Data are means from two replications.

<sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 1.7571.

These data indicate that levels of *Campylobacter* spp. in the troughs were relatively low. In this study, the prevalence of *Campylobacter* spp. was not detected from trough samples in the battery-cage system during weeks 1, 3, 5 and 6 but was detected in weeks 2 and 4 at 66.67% (96|144 samples) and 47.22% (68|144 samples) respectively (Table 3.4). *Campylobacter* spp. was not detected from trough samples in the cage-free system in weeks 1, 5, and 6 but was detected in weeks 2, 3 and 4 at 16.67% (24|144 samples), 25.0% (36|144 samples) and 17.36% (25|144 samples) respectively (Table 3.4). Additionally, the specific bacterium *C. jejuni* was detected from trough samples in weeks 3 and 4 in the cage-free system at 25% (36|144 samples) and 11.81% (17|144 samples) respectively (Table 3.4). The prevalence of *C. jejuni* from trough samples was not detected in the battery-cage system.

Table 3.4 The prevalence of *Campylobacter* spp. and *C. jejuni* from trough samples from the battery-cage and the cage-free systems from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 144)		No. (%) of positive samples of <i>C. jejuni</i> (n = 144)	
	Battery-cage	Cage-free	Battery-cage	Cage-free
1	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
2	96 (66.67) <sup>a</sup>	24 (16.67) <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>
3	ND <sup>a</sup>	36 (25.00) <sup>b</sup>	ND <sup>a</sup>	36 (25.00) <sup>b</sup>
4	68 (47.22) <sup>a</sup>	25 (17.36) <sup>b</sup>	ND <sup>a</sup>	17 (11.81) <sup>a</sup>
5	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
6	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>

ND = Non detectable. Data are means from two replications. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different, P<0.05. SEM for *Campylobacter* spp. = 0.0661; SEM for *C. jejuni* = 0.0425.

As is the case for drinking water, feed and enclosures described above, troughs can be a source for *Campylobacter* cross-contamination. Once again, the typical route appears to be from feces to beak. This problem may be more prevalent in the cage-free system than in the battery-cage system. However, good sanitation practices are important in the battery-cage and the cage-free systems. Again, the application of Nolvasan Solution is appropriate for routine cleaning and disinfecting in small-scale poultry operations.

### 3.3.3 Live Broilers

In chickens, *Campylobacter* colonizes the mucus overlying the epithelial cells primarily in the cecum and the small intestine. For sampling purposes, *Campylobacter* spp. is collected from feces (Newell and Fearnley, 2003). In this study, 150 individual chickens (75 from the battery-cage system and 75 from the cage-free system) were sampled for the presence of

*Campylobacter* spp. and *C. jejuni* for two replications. Using these protocols *Campylobacter* spp. and *C. jejuni* were successfully isolated. Each replication period extended for a period of six weeks, the time it takes for commercial farmers to raise, harvest and bring their birds to market.

From enumeration tests, counts of *Campylobacter* spp. steadily increased from week 1 to week 6 in both the battery-cage and the cage-free systems. The counts of *Campylobacter* spp. in the battery-cage system increased from an initial value of 1.70 log CFU/ml in week one to a maximum value of 4.91 log CFU/ml in week six (Figure 3.5). In the cage-free system the initial value of 0 log CFU/ml in week one increased to a maximum value of 5.31 log CFU/ml in week six (Figure 3.5).

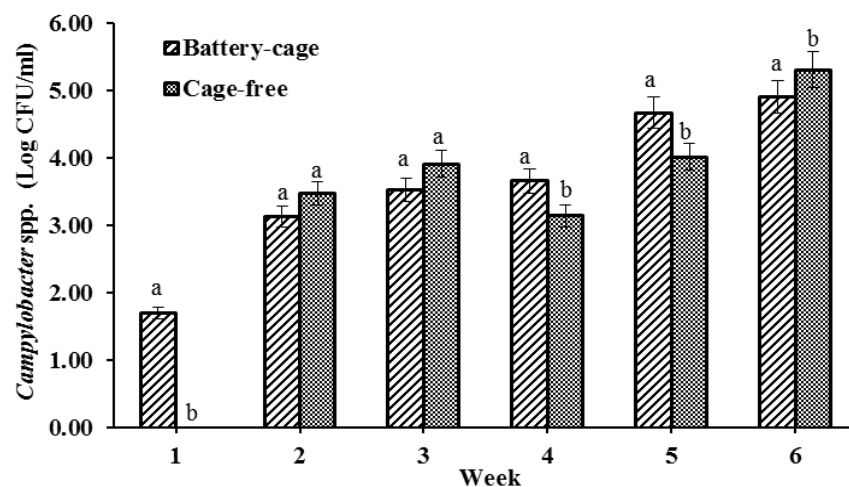


Figure 3.5 *Campylobacter* spp. bacterial counts in live broilers from the battery-cage and the cage-free systems from weeks 1 through 6. Data are means from two experiments. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 3.2807.

The counts of *Campylobacter* spp. in the cage-free system were materially higher than those of the battery-cage system ( $P < 0.05$ ) (Figure 3.5). As noted above, the presence of *Campylobacter* spp. in drinking water, feed, enclosures and troughs was elevated in the samples

taken in the cage-free system compared to those taken in the battery-cage system. As these are probable vectors in the cross-contamination of *Campylobacter* spp., it is reasonably postulated that these are contributing factors in the elevated levels of *Campylobacter* spp. contamination found in the cage-free birds.

Prevalence tests were also performed during the six weeks of sampling. For each of the test weeks, the prevalence of *Campylobacter* spp. in individual chickens from the battery-cage system ranged from 5.33% (8|150 samples) to 83.33% (125|150 samples) (Table 3.5).

Table 3.5 The prevalence of *Campylobacter* spp. and *C. jejuni* in broilers from the battery-cage and the cage-free systems from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 150)		No. (%) of positive samples of <i>C. jejuni</i> (n = 150)	
	Battery-cage	Cage-free	Battery-cage	Cage-free
1	8 (5.33) <sup>a</sup>	ND <sup>b</sup>	8 (5.33) <sup>a</sup>	ND <sup>b</sup>
2	86 (57.33) <sup>a</sup>	48 (32.00) <sup>b</sup>	66 (44.00) <sup>a</sup>	48 (32.00) <sup>b</sup>
3	125 (83.33) <sup>a</sup>	140 (93.33) <sup>b</sup>	28 (18.67) <sup>a</sup>	106 (70.67) <sup>b</sup>
4	115 (76.67) <sup>a</sup>	123 (82.00) <sup>b</sup>	35 (23.33) <sup>a</sup>	35 (23.33) <sup>a</sup>
5	75 (50.00) <sup>a</sup>	60(40.00) <sup>b</sup>	75 (50.00) <sup>a</sup>	60 (40.00) <sup>b</sup>
6	39 (26.00) <sup>a</sup>	56 (37.33) <sup>b</sup>	39 (26.00) <sup>a</sup>	56 (37.33) <sup>b</sup>

ND = Non detectable. Data are means from two experiments. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different, P<0.05. SEM for *Campylobacter* spp. = 0.0228; SEM for *C. jejuni* = 0.0266.

From the cage-free system, the prevalence of *Campylobacter* spp. ranged from 0% (0|150 samples) to 93.33% (140|150 samples) (Table 3.5). These results indicated that the prevalence of *Campylobacter* spp. in individual broilers peaked during the third week. Specifically, there was a prevalence of 93.33% (140|150 samples) in the cage-free birds and 83.33% (125|150 samples) in



the battery-cage birds (Table 3.5). Other studies also have shown that the prevalence of *Campylobacter* spp. peaks and then declines over time. For example, Sahin et al. (2003) found similar results in their study of commercial chickens. Northcutt et al. (2003) posited that this decline might be due to maturation of antibodies passed from hens to their chicks.

From additional testing via hippurate hydrolysis, the prevalence of the specific bacterium *C. jejuni* was detected. Specifically, the prevalence ranged from 5.33% (8|150 samples) to 50.0% (75|150 samples) in the battery-cage system and from 0% (0|150 samples) to 70.67% (106|150 samples) in the cage-free system (Table 3.5). The prevalence of *C. jejuni* in week 6 was significantly higher ( $P<0.05$ ) in the cage-free system (37.33%; 56|150 samples) than in the battery-cage system (26.0%; 39|150 samples) (Table 3.5). As stated previously, the elevated prevalence of *C. jejuni* in the drinking water, feed, enclosures and troughs in the cage-free system are probable contributing factors in the elevated levels of *C. jejuni* in those birds. Again, the application of Nolvasan Solution should substantially mitigate such cross-contamination.

These results provide valuable base-line data concerning the presence and extent of *Campylobacter* in broilers raised in a small-scale poultry setting. This study next explores an avenue whereby *Campylobacter* infection in broilers is mitigated through the use of the antibiotic sulfadimethoxine. The details of these experiments are the subject of Chapter 4 of this dissertation.

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## CHAPTER 4

### THE EFFECTS OF SULFADIMETHOXINE ADMINISTERED TO CONTROL *C. JEJUNI* INFECTION IN SMALL-SCALE BROILER OPERATIONS

#### 4.1 INTRODUCTION

*Campylobacter* spp. particularly *Campylobacter jejuni* is a common pathogenic cause of human foodborne gastroenteritis worldwide (Adak et al., 2005). Annually, approximately 400 million cases of *Campylobacter*-associated gastroenteritis occur (Rao et al., 2001; Van et al., 2001) with an economic impact estimated in excess of \$2 billion (Allos, 2001). The European Union (EU), reported 214,268 confirmed cases of campylobacteriosis in 2012 at a cost of 2.4 billion Euros (European Food Safety Authority, 2014). Havelaar et al. (2005) estimated that in the Netherlands with approximately 80,000 cases of gastroenteritis per year, the cost of illness caused by campylobacteriosis is about 21 million Euros.

In the United States, *Campylobacter* spp. is responsible for an estimated 2.1-2.4 million cases of foodborne illnesses each year (Blaser, 1997; Altekruuse et al., 1999; Friedman et al., 2000; Samuel et al., 2004; Scallan et al., 2011) resulting in 13,000 hospitalizations, 100 deaths and an estimated cost of over \$1 billion annually (Altekruuse et al., 1999; Samuel et al., 2004; Anonymous, 2010). In 2013, FoodNet identified 19,056 laboratory-confirmed cases of human *Campylobacter* infection. The number of infections and incidence per 100,000 populations by *Campylobacter* are 6,621 and 13.82 respectively. Of these infected populations, 1,010 people (15%) were hospitalized and 12 people (0.2%) died from food contaminated by *Campylobacter* (FoodNet, 2013).

Approximately 70% of human illnesses due to *Campylobacter* spp. are caused by the consumption or handling of raw or undercooked poultry (Friedman et al., 2000; Mead et al., 1999). Additionally, *Campylobacter* spp. can be transmitted via contact with infected animals or

their feces. Many animals carry *Campylobacter* spp. asymptotically and shed the bacterium in their feces. Poultry, particularly broiler chickens, also frequently harbor the bacterium.

Because of the threat to public health, serious efforts are being made to prevent the colonization and spread of *Campylobacter* spp. and *C. jejuni* in poultry production (Allos, 2001; Friedman et al., 2004; Keener et al., 2004). A reduction in numbers of *Campylobacter* spp. and *C. jejuni* in poultry, production can lead to a corresponding reduction in human infections. Quantitative risk assessment models have indicated that a reduction of 2 log units on a broiler carcass could result in 30 times less prevalence of campylobacteriosis (Rosenquist et al., 2003). Therefore, reduction or elimination of *Campylobacter* spp. and *C. jejuni* in the poultry reservoir is an essential consideration in the control of this food safety problem.

Although there are multiple levels at which *Campylobacter* spp. and *C. jejuni* contamination can be targeted, on-farm control of *Campylobacter* spp. and *C. jejuni* has the greatest impact because the living poultry intestine is the primary amplification point for *Campylobacter* throughout the food chain (Wagenaar et al., 2006; Wagenaar et al., 2008). Therefore, the use of various antimicrobial therapies to control *Campylobacter* spp. and *C. jejuni* infection in poultry production is worthy of exploration.

Antimicrobial therapy is a potentially important tool in reducing the prevalence and enumeration of *C. jejuni* in poultry. Several studies have addressed the use and efficacy of antibiotics on an array of poultry infections including *Campylobacter* spp. and *C. jejuni*. For the most part, the results are mixed. For example, the *in vivo* study by Carvalho et al. (2010) found that a three-phase lytic cocktail administered to chickens resulted in a 2 log CFU/g reduction in *C. jejuni*. In another *in vivo* study using turkeys, Scupham et al. (2010) found that the administration of enrofloxacin, neomycin and vancomycin resulted in a respective decrease of

1, 2 and 4 log CFU/g in *C. jejuni*. Additionally, Robyn et al. (2013) attempted to inhibit *C. jejuni* in chickens through the administration of live bacterium *Enterococcus faecalis*. As they reported, this bacterium failed to inhibit the growth of *C. jejuni*.

Sulfadimethoxine is an antibiotic administered in commercial poultry production to treat a variety of infections (Food and Drug Administration, 2013). These include the respiratory illnesses, coccidiosis, fowl cholera, and coryza (Wang et al., 2012). Sulfadimethoxine inhibits the bacterial synthesis of folic acid (pteroylglutamic acid) from para-aminobenzoic acid (Vree and Hekster, 1987).

To date, there have been no *in vivo* studies on the efficacy of sulfadimethoxine in the control of *C. jejuni* in broilers in small-scale poultry operations. Although largely unknown, the positive potential effects of sulfadimethoxine antibiotic treatment to control *C. jejuni* infection in chickens should be explored.

The purpose of this study is to determine the effects of sulfadimethoxine antibiotic on the enumeration of *Campylobacter* spp. and the prevalence on both *Campylobacter* spp. and *C. jejuni* in growing broilers. Also, this study will examine the effects of sulfadimethoxine antibiotic treatments on the enumeration of *Campylobacter* spp. and prevalence of *Campylobacter* spp. and *C. jejuni* from the likely sources of cross-contamination including water, feed, enclosures and troughs.

## **4.2 MATERIALS AND METHODS**

### **4.2.1 Sample Collection**

#### **4.2.1.1 Broiler Production**

Two treatments with two replications each using 300 broiler chickens (Ross × Ross) obtained from a commercial hatchery were used. These experiments were conducted from

January 2014 to May 2014. Birds were housed in a controlled environment and maintained in Petersime<sup>®</sup> Battery Cages (32°C) with raised wire flooring. Each cage was divided into 12 pens of equal size (29.4" x 39" x 9.5"). Each pen housed twenty-five birds. Feed was procured from the Texas Farm Products Company. This feed contains 18% protein chick grower crumbles and no antibiotics. Individual water and feed troughs were provided for each pen and feed was provided free-choice.

Birds were allotted to one of two treatments: 1) control (tap water) and 2) drinking water + 0.05% (wt/vol) sulfadimethoxine (Durvet Inc., Blue Springs, Missouri). Drinking water was refreshed every day in both treatment groups. The pH value of drinking water was determined with a probe electrode portable meter (Beckman 265 pH/ temp/ mV/ Meter). Calibration of the pH meter was accomplished using pH 7 and pH 4 standardization buffers. Each week, 150 individual broilers were randomly sampled using Sterile Rayon Tipped Swabs. The sample swabs were placed in a tube containing 3 ml of sterile tryptone soya broth (TSB) for further analysis.

#### 4.2.1.2 Environment Samples

Two replications, one hundred forty-four samples were randomly collected from drinking water, feed and equipment (enclosures and troughs). A 3-5 g feed sample and 5-10 ml drinking water sample was collected from each feed and water trough and placed in sterile bottles. Surface areas of enclosures and troughs were swabbed and immediately placed into tubes containing 3 ml of TSB for further analysis.

Chicken feces and environment samples were evaluated from January 2014 to May 2014. All samples were collected using aseptic techniques in sterile containers and were transported to the lab and analyzed at weeks 1, 2, 3, 4, 5 and 6. Quantitative concentration of *Campylobacter*



spp. was determined using the method described by Corry et al. (2003). From the samples, *Campylobacter* spp. was isolated using Latex Agglutination tests (Moore et al., 2005; Miller et al., 2008).

#### **4.2.2 Bacterial Isolation and Identification**

Immediately upon arrival in the laboratory, the swab samples were whirl-mixed in a shaker incubator (New Brunswick Scientific Excella™ E24/E24R Temperature-Controlled Benchtop Shaker) for approximately 1 h at 37°C and then mixed with a vortexer for 2 min to release the bacteria. Each 0.1 ml of swab sample was aseptically transferred and directly streaked onto modified charcoal cefoperazone deoxycholate agar (mCDDA). The inoculated plates were then incubated at 42°C for 48 h in a microaerophilic environment (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>). *Campylobacter* spp. was verified by Latex agglutination tests with a Microgen M46 *Campylobacter* Assay Kit (Microgen Bioproducts Ltd., Camberley, Surrey, United Kingdom). In addition, a hippurate hydrolysis test was performed to confirm *C. jejuni* (Hwang and Ederer, 1975).

#### **4.2.3 *Campylobacter* Latex Agglutination Kit**

The Microgen Kit is composed of F46a (Test Latex Reagent): latex particles coated with rabbit antibodies to *Campylobacter* antigens, F46b (Control Latex Reagent): latex particles coated with non-specific rabbit immunoglobulins, F46c (Positive Control): suspension of inactivated *Campylobacter* antigens reactive with test latex reagent and non-reactive with control latex reagent, and F40 (0.85% Isotonic Saline).

All reagents were allowed to reach room temperature and gently shaken to ensure a homogeneous suspension. One drop (50 µL) of isotonic saline (F40) was dispensed to each of the two ovals of the agglutination slide. An inoculating loop was used to remove several colonies

with *Campylobacter*-like morphology. Bacteria were mixed into each of the two drops of isotonic saline (F40) on the slide to form an even suspension. One drop (50 µL) of Control Latex reagent (F46b) was added to one of the bacterial suspensions on the slide. One drop (50 µL) of Test Latex Reagent (F46a) was similarly dispensed to the other bacterial suspension. The bacterial suspensions were mixed with latex reagents using a mixing stick with the Control Latex Reagent (F46b). The mixtures were spread to the edges of the oval areas. The slide was gently rocked to keep the fluid suspensions in constant movement for 2 min to produce agglutination. Strength of the reaction is variable and was assessed according to the following: + reaction: fine, but readily discernible granularity against a milky background, ++ reaction: coarse granularity against a milky background, and +++ reaction: heavy clumping of particles around the periphery of the test oval, against a clear background.

#### **4.2.4 Hippurate Hydrolysis Test**

This test is used to determine whether a microorganism, by action of the enzyme hippurate hydrolase, can hydrolyze sodium hippurate to benzoic acid and glycine. The glycine end product is detected by the addition of ninhydrin reagent and through a complex reaction forms a deep purple color that can be easily visualized for the identification of *C. jejuni*.

A 1% aqueous solution of sodium hippurate was prepared and dispensed in 0.4 ml amounts into 0.2 ml centrifuged tubes. Inoculum from 24 h of *Campylobacter* growth of the test organism was emulsified into a tube of the hippurate solution and incubated in a 37°C for 2 h. After incubation, 0.2 ml of the ninhydrin solution was carefully added down the side of the tube to form an overlay over the cell mixture so not to mix the solutions. Samples were incubated for 10 minutes and observed. A deep purple-blue result indicated a positive test for *C. jejuni*. A colorless result indicated a negative result for the presence of *C. jejuni*.

#### **4.2.5 Statistical Analysis**

Statistical procedures were performed using SAS Windows (SAS Institute, 2003). A block design with two replications was randomly assigned to the control and experimental groups of chicken production.

All calculations were performed with Proc GLM procedures (SAS, 2003) using  $P = 0.05$  for significance of Least Squares Means and simple correlation coefficients among variables with a model of the antibiotic sulfadimethoxine administration in chicken production and period time testing. Upon the detection of treatment differences, specific comparisons between treatment means at that time point were made with the PDIFF option of LSMEANS.

### **4.3 RESULTS AND DISCUSSION**

#### **4.3.1 Environment Samples**

##### **4.3.1.1 Drinking Water**

The optimum pH growth environment for *Campylobacter* spp. is 6.5–7.5. However, cultures can survive at pH levels between 4.9 and 9.0. At pH levels below 4.0, cells rapidly die, especially under refrigeration temperatures (ICMSF, 1996). In this study, the initial pH value of the non-treated drinking water (control treatment) was 7.97 and the drinking water + 0.05% sulfadimethoxine (antibiotic treatment) was at pH 8.30.

Over the six week period, *Campylobacter* spp. counts from the control treatment ranged from 0-2.56 log CFU/ml. In the antibiotic treatment, the results ranged from 0-2.10 log CFU/ml (Figure 4.1).

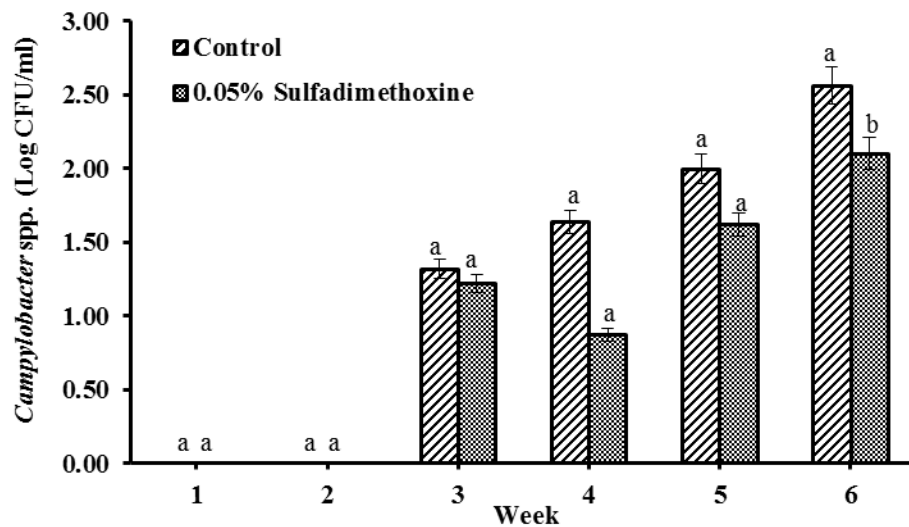


Figure 4.1 *Campylobacter* spp. bacterial counts in drinking water samples from the control and antibiotic treatments from weeks 1 through 6. Data are means from two experiments.

<sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 1.1451.

The presence of *Campylobacter* spp. was not detected in drinking water samples in weeks 1 or 2. However, the presence of *Campylobacter* spp. was detected in week 3 and continued to increase during the course of the experiment in both the control and antibiotic treatments. However, the levels of *Campylobacter* spp. were slightly higher ( $P > 0.05$ ) in the control treatment when compared to the antibiotic treatment (Figure 4.1).

In the antibiotic treatment, *Campylobacter* spp. increased by 0.88 log CFU/ml whereas, in the control treatment, it increased by 1.24 log CFU/ml from weeks 3 to 6. Subsequently, these results clearly show that the enumeration of *Campylobacter* spp. in water samples was significantly higher ( $P < 0.05$ ) in the control treatment than in the antibiotic treatment at week 6 (Figure 4.1). These results suggest that the application of a 0.05% concentration of sulfadimethoxine can reduce the presence of *Campylobacter* spp. in the drinking water, thereby mitigating cross-contamination.

The prevalence of *Campylobacter* spp., verified by latex agglutination tests, ranged from 0% to 50% (72|144 samples) in the control treatment and ranged from 0% to 41.67% (60|144 samples) in the antibiotic treatment over the six-week period (Table 4.1).

Table 4.1 The prevalence of *Campylobacter* spp. and *C. jejuni* in drinking water samples from the control and antibiotic treatments from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 144)		No. (%) of positive samples of <i>C. jejuni</i> (n = 144)	
	Control	0.05% Sulfadimethoxine	Control	0.05% Sulfadimethoxine
1	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
2	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
3	48 (33.33) <sup>a</sup>	48 (33.33) <sup>a</sup>	24 (16.67) <sup>a</sup>	12 (8.33) <sup>b</sup>
4	36 (25.00) <sup>a</sup>	24 (16.67) <sup>b</sup>	12 (8.33) <sup>a</sup>	12 (8.33) <sup>a</sup>
5	60 (41.67) <sup>a</sup>	36 (25.00) <sup>b</sup>	36 (25.00) <sup>a</sup>	12 (8.33) <sup>b</sup>
6	72 (50.00) <sup>a</sup>	60 (41.67) <sup>b</sup>	60 (41.67) <sup>a</sup>	40 (27.78) <sup>b</sup>

ND = Non detectable. Data are means from two experiments. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different, P<0.05. SEM for *Campylobacter* spp. = 0.0189; SEM for *C. jejuni* = 0.0194.

The presence of *Campylobacter* spp. was not detected in either the control or antibiotic water samples in weeks 1 or 2. This suggests that little or no *Campylobacter* spp. cross-contamination had occurred. However, the presence of *Campylobacter* spp. in the drinking water was initially detected in both the control and antibiotic treatments in week 3. In week 3, the prevalence of *Campylobacter* spp. was measured at 33.33% (48|144 samples) in both the control and antibiotic treatments (Table 4.1).

In week 4, the prevalence of *Campylobacter* spp. declined in both the control and antibiotic treatments. Specifically, the prevalence was measured at 16.67% (24|144 samples) in

the antibiotic treatment and at 25% (36|144 samples) in the control treatment (Table 4.1). At week 5, the prevalence of *Campylobacter* spp. increased to 41.67%, (60|144 samples) in the control treatment and to 25% (36|144 samples) in the antibiotic treatment. At week 6, the control treatment exhibited a significantly higher prevalence of *Campylobacter* spp. ( $P<0.05$ ) at 50% (72|144 samples) than in the antibiotic treatment at 41.67% (60|144 samples) (Table 4.1). These results demonstrate that the prevalence of *Campylobacter* spp. was significantly less in the treated drinking water than in the control.

The prevalence of *C. jejuni* as determined through hippurate hydrolysis in drinking water samples ranged from 0% (0|144 samples) to 41.67% (60|144 samples) in the control treatment and from 0% (0|144 samples) to 27.78% (40|144 samples) in the antibiotic treatment over the six-week period of testing (Table 4.1). At weeks 1 and 2, *C. jejuni* was not detected. At week 3, the prevalence of *C. jejuni* was measured at 16.67% (24|144 samples) in the control treatment and 8.33% (12|144 samples) in the antibiotic treatment (Table 4.1).

The prevalence of *C. jejuni* was measured at 8.33% (12|144 samples) in both the control and antibiotic treatments at week 4 (Table 4.1). At week 5, the prevalence of *C. jejuni* increased to 25.0%, (36|144 samples) in the control treatment but it was stable at 8.33% in the antibiotic treatment (Table 4.1). At week 6, the control treatment exhibited a significantly higher prevalence of *C. jejuni* ( $P<0.05$ ) at 41.67% (60|144 samples) than in the antibiotic treatment at 27.78% (40|144 samples) (Table 4.1). These results indicated that the prevalence of *C. jejuni* in the antibiotic treatment decreased by 28.80% (20|144 samples) as compared to the control in week 6 (Table 4.1). These results suggest that the administration of 0.05% sulfadimethoxine reduces the prevalence of *C. jejuni* in drinking water. In a small-scale poultry setting, this practice might mitigate cross contamination of the bacterium.

#### 4.3.1.2 Feed

In this study, fresh feed from each bag was tested for *Campylobacter* spp. and *C. jejuni* before it was fed to the broilers. Neither *Campylobacter* spp. nor *C. jejuni* was detected. Subsequently, *Campylobacter* spp. counts from feed samples ranged from 0-1.80 log CFU/ml in the control treatment and from 0-1.30 log CFU/ml in the antibiotic treatment over the six-week test period (Figure 4.2). *Campylobacter* spp. was not detected in either the control or antibiotic treatments in weeks 1, 2, 4, and 5. However, the presence of *Campylobacter* spp. was detected in both the antibiotic and control treatments in week 3. Specifically, *Campylobacter* spp. was detected at a concentration of 1.80 log CFU/ml in the control treatment and at 0.82 log CFU/ml in the antibiotic treatment. These calculations suggest a significant difference ( $P < 0.05$ ) between the control and antibiotic treatments. However, at week 6 the difference was not significant ( $P > 0.05$ ) as the concentration of *Campylobacter* spp. in the control was determined to be 0.22 log CFU/ml and 0.00 log CFU/ml in the antibiotic treatment (Figure 4.2). The reason for the *Campylobacter* spp. spike in week 3 is uncertain. This spike might be the result of a simple anomaly or it might represent a one-time event.

The prevalence of *Campylobacter* spp. in the feed samples ranged from 0% to 50.0% (72|144 samples) in the control treatment and from 0-16.67% (24|144 samples) in the antibiotic treatment (Table 4.2). As was the case in the enumeration study, *Campylobacter* spp. was not detected in weeks 1, 2, 4, and 5 in either the control or antibiotic treatments. The prevalence of *Campylobacter* spp. spiked at week 3.

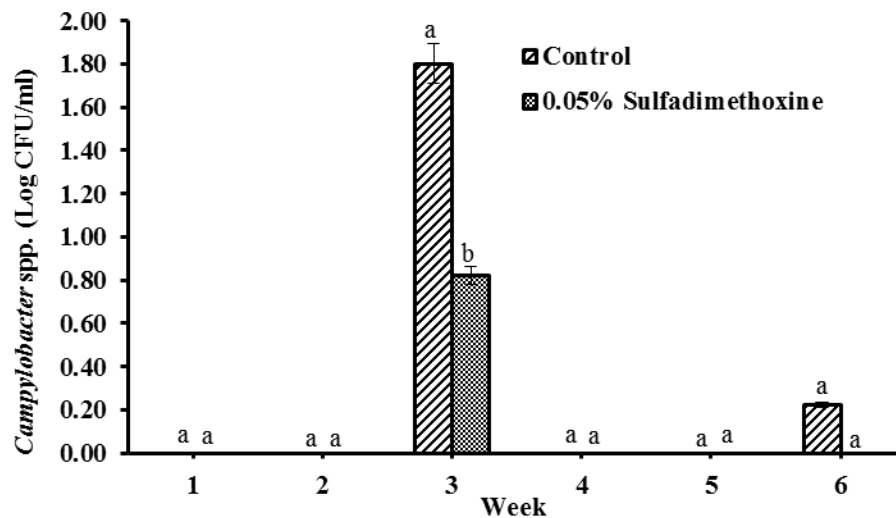


Figure 4.2 *Campylobacter* spp. bacterial counts in feed samples from the control and antibiotic treatments from weeks 1 through 6. Data are means from two experiments. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 0.4294.

Table 4.2 The prevalence of *Campylobacter* spp. and *C. jejuni* in feed samples from the control and antibiotic treatments from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 144)		No. (%) of positive samples of <i>C. jejuni</i> (n = 144)	
	Control	0.05% Sulfadimethoxine	Control	0.05% Sulfadimethoxine
1	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
2	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
3	72 (50.00) <sup>a</sup>	24 (16.67) <sup>b</sup>	36 (25.00) <sup>a</sup>	12 (8.33) <sup>b</sup>
4	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
5	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
6	12 (8.33) <sup>a</sup>	ND <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>

ND = Non detectable. Data are means from two experiments. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM for *Campylobacter* spp. = 0.0191; SEM for *C. jejuni* = 0.0128.



Specifically, in the control treatment, prevalence was determined at 50% (72|144 samples) and at 16.67% (24|144 samples) in the antibiotic treatment (Table 4.2).

At week 6, the prevalence of *Campylobacter* spp. at 8.33% (12|144 samples) in the control treatment was significantly different ( $P < 0.05$ ) than that of the antibiotic treatment at 0.00% (0|144 samples). As was the case in the enumeration test, the prevalence test shows fewer spikes. For the reasons stated above, an explanation of this spike is uncertain.

As was the case in the previous tests, the prevalence of the specific bacterium, *C. jejuni* spiked in week 3 in both treatments. However, the prevalence of 25% (36|144 samples) in the control was significantly higher ( $P < 0.05$ ) than in the antibiotic treatment 8.33% (12|144 samples) (Table 4.2). *C. jejuni* was not detected in weeks 1, 2, 4, 5, and 6 (Table 4.2). Although the spike in week 3 is consistent with the previous tests, the reason for this remains uncertain. Therefore, the use of the antibiotic might still be useful in controlling these kinds of spikes that could lead to a significant cross-contamination event.

#### 4.3.1.3 Enclosures and Troughs

In this study, enclosures and troughs were sampled for enumeration of *Campylobacter* spp. and for the prevalence of *Campylobacter* spp. and *C. jejuni*. Of the 144 samples taken from each of the control and experimental groups during the six-week period of testing, none were positive for *Campylobacter* spp. or *C. jejuni*.

As stated previously, enclosures and troughs were tested because they might harbor *Campylobacter* spp. and, therefore, contribute to cross-contamination during poultry production. Because the control samples showed no incidence of *Campylobacter* spp. it appears that enclosures and troughs are not a significant point of bacterial refuge. Therefore, enclosures and

troughs do not appear to be a material concern in controlling *Campylobacter* spp. in small-scale poultry production.

#### 4.3.2 Live Broilers

##### 4.3.2.1 Enumeration Testing

Enumeration test were performed at the McNeese State University Agricultural Sciences laboratory for two replications on 300 chickens from January through May 2014. For each replication, 150 individual chickens were used. The control and antibiotic treatments each consisted of 75 birds.

From these enumeration tests, counts of *Campylobacter* spp. steadily increased from week 1 through week 6 in both the control and antibiotic treatments (Figure 4.3). The counts of *Campylobacter* spp. in the control treatment increased from an initial value of 3.58 log CFU/ml in week one to a maximum value of 6.05 log CFU/ml in week six. This represents a total increase of 2.47 log CFU/ml during the course of the experiment. In the antibiotic treatment the initial value of 3.44 log CFU/ml in week one increased to a maximum value of 5.12 log CFU/ml in week six (Figure 4.3). This represents a total increase of 1.68 log CFU/ml.

There was no significant difference in the enumeration of *Campylobacter* spp. in the antibiotic treatment ( $P>0.05$ ) and the control treatment in weeks 1 through 5. However, the enumeration of *Campylobacter* spp. was significantly higher in the control treatment than the antibiotic treatment ( $P<0.05$ ) at week 6 (Figure 4.3).

For the overall experiment, the enumeration of *Campylobacter* spp. in the antibiotic treatment was lower than in the control treatment (Figure 4.3). These results suggest that the antibiotic sulfadimethoxine, as applied in this experiment can reduce the enumeration of *Campylobacter* spp. in the broilers.

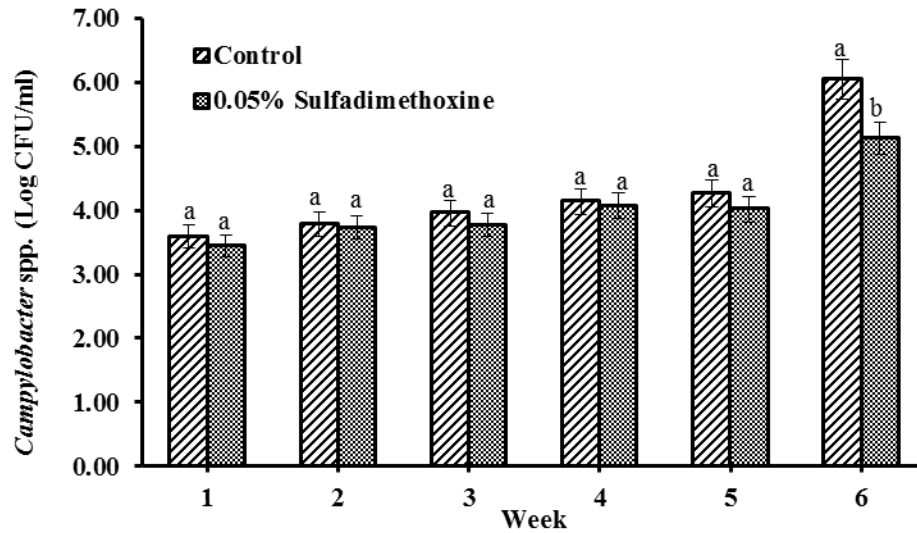


Figure 4.3 Enumeration results on *Campylobacter* spp. in live broilers from the control and antibiotic treatments from weeks 1 through 6. Data are means from two experiments.

<sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM = 4.9738.

#### 4.3.2.2 Prevalence Testing

##### 4.3.2.2.1 *Campylobacter* spp.

Prevalence tests for *Campylobacter* spp. were performed for two replications on 300 chickens from January through May 2014. For each replication, 150 individual chickens were used. The control and experimental treatments each consisted of 75 birds.

For each of the test weeks, the prevalence of *Campylobacter* spp. in individual broilers from the control treatment ranged from 37.33% (56|150 samples) to 66.67% (100|150 samples). From the antibiotic treatment, the prevalence ranged from 13.33% (20|150 samples) to 65.33% (98|150 samples) (Table 4.3). At week 1, the prevalence of *Campylobacter* spp. was significantly lower in the antibiotic treatment ( $P < 0.05$ ) than in the control treatment (Table 4.3). In weeks 2 and 3, the prevalence of *Campylobacter* spp. declined by 51.00% (34|150 samples) in the antibiotic treatment but increased by 36.00% (24|150 samples) in the control treatment.

Table 4.3 The prevalence of *Campylobacter* spp. and *C. jejuni* in live broilers from the control and antibiotic treatments from weeks 1 through 6.

Week	No. (%) of positive samples of <i>Campylobacter</i> spp. (n = 150)		No. (%) of positive samples of <i>C. jejuni</i> (n = 150)	
	Control	0.05% Sulfadimethoxine	Control	0.05% Sulfadimethoxine
1	62 (41.33) <sup>a</sup>	46 (30.67) <sup>b</sup>	62 (41.33) <sup>a</sup>	38 (25.33) <sup>b</sup>
2	56 (37.33) <sup>a</sup>	54 (36.00) <sup>a</sup>	50 (33.33) <sup>a</sup>	50 (33.33) <sup>a</sup>
3	80 (53.33) <sup>a</sup>	20 (13.33) <sup>b</sup>	68 (45.33) <sup>a</sup>	20 (13.33) <sup>b</sup>
4	80 (53.33) <sup>a</sup>	78 (52.00) <sup>a</sup>	72 (48.00) <sup>a</sup>	68 (45.33) <sup>a</sup>
5	64 (42.67) <sup>a</sup>	62 (41.33) <sup>a</sup>	64 (42.67) <sup>a</sup>	62 (41.33) <sup>a</sup>
6	100 (66.67) <sup>a</sup>	98 (65.33) <sup>a</sup>	84 (56.00) <sup>a</sup>	75 (50.00) <sup>b</sup>

Data are means from two experiments. <sup>a,b</sup>Treatment means with different superscripts for the same week are significantly different,  $P < 0.05$ . SEM for *Campylobacter* spp. = 0.0344; SEM for *C. jejuni* = 0.0366.

In week 3, the prevalence of *Campylobacter* spp. was significantly higher in the control treatment than the antibiotic treatment ( $P < 0.05$ ) (Table 4.3). Specifically, there was a 13.33% (20|150) incidence in the antibiotic treatment and a 53.33% (80|150) incidence in the control treatment. These finding showed that the antibiotic sulfadimethoxine can reduce the prevalence of *Campylobacter* spp. in broilers especially in this week (Table 4.3).

In week 4, the prevalence of *Campylobacter* spp. increased to 52.0%, (78|150 samples) in the antibiotic treatment but it was unchanged at 53.33% (80|150 samples) in the control treatment. In week 5, the prevalence of *Campylobacter* spp. declined somewhat in both treatments. Specifically, the prevalence was measured at 41.33% (62|150 samples) in the antibiotic treatment and at 42.67% (64|150 samples) in the control treatment (Table 4.3). In week 6, the prevalence of *Campylobacter* spp. in the control treatment was measured at

66.67% (100|150 samples) and in the antibiotic treatment was measured at 65.33% (98|150 samples) (Table 4.3). Overall, for the six-week period of testing, the prevalence of *Campylobacter* spp. in the antibiotic treatment was lower ( $P<0.05$ ) than in the control treatment (Table 4.3).

#### 4.3.2.2.2 *C. jejuni*

Prevalence tests for *C. jejuni* were performed for two replications on 300 chickens from January through May 2014. For each replication, 150 individual chickens were used. The control and antibiotic treatments each consisted of 75 birds.

In this study, the overall prevalence of *C. jejuni* in the control treatment ranged from 33.33% (50|150 samples) to 56.00% (84|150 samples) and from 13.33% (20|150 samples) to 50.00% (75|150 samples) in the antibiotic treatment (Table 4.3).

In week 1, the prevalence of *C. jejuni* was significantly higher ( $P<0.05$ ) in the control treatment at 41.33% (62|150 samples) than in the antibiotic treatment at 25.33% (38|150 samples) (Table 4.3). In week 2, the prevalence of *C. jejuni* was the same in both control and antibiotic treatments at 33.33% (50|150 samples) (Table 4.3). In week 3, the prevalence of *C. jejuni* declined in the antibiotic treatment to 13.33% (20|150 samples) whereas, it increased to 45.33% (68|150 samples) in the control treatment. These values represent a significant difference ( $P<0.05$ ) (Table 4.3).

In week 4, the prevalence of *C. jejuni* was at 45.33% (68|150 samples) in the antibiotic treatment and at 48.00% (72|150 samples) in the control treatment (Table 4.3). These values are not significantly different ( $P>0.05$ ) (Table 4.3). In week 5, the prevalence of *C. jejuni* was at 41.33% (62|150 samples) in the antibiotic treatment and at 42.67% (64|150 samples) in the control treatment (Table 4.3). These values are not significantly different ( $P>0.05$ ) (Table 4.3). In

week 6, the prevalence of *C. jejuni* was significantly higher ( $P<0.05$ ) in the control treatment at 56.00% (84/150 samples) than in the antibiotic treatment at 50.00% (75/144 samples) (Table 4.3). For the overall experiment, the prevalence of *C. jejuni* in the antibiotic treatment was significantly lower ( $P<0.05$ ) than in the control treatment (Table 4.3). These results suggest that the antibiotic sulfadimethoxine, as applied in this experiment can reduce the prevalence of *C. jejuni* in the broilers.

#### 4.3.3 Summary

This study indicates the use of antibiotic sulfadimethoxine can reduce the enumeration of *Campylobacter* spp. and the prevalence of *Campylobacter* spp. and *C. jejuni* in small-scale poultry farming. This is the first study in which sulfadimethoxine has been used in an in vivo setting to control *Campylobacter*. Therefore, this study supplies the first such data in the literature.

Results from other studies whose aim was to control *C. jejuni* in poultry are mixed. For example, the study by Robyn et al. (2013) attempted to inhibit *C. jejuni* in chickens through the administration of live bacterium *Enterococcus faecalis*. As they reported, this bacterium failed to inhibit the growth of *C. jejuni*. In a second study, Carvalho et al. (2010) found that bacteriophage lytic cocktail administered to chickens resulted in a 2 log CFU/ml reduction in the enumeration of *C. jejuni*. In a third study using turkeys, the administration of the antibiotics enrofloxacin, neomycin and vancomycin resulted in a respective decrease of 1, 2 and 4 log CFU/ml of *C. jejuni* (Scupham et al., 2010). For purposes of comparison, the present study found that the use of sulfadimethoxine resulted in a decrease of 0.93 log CFU/ml in the experimental group as compared to the control group. Therefore, the positive results from this study compare favorably with the previously mentioned second and third studies.

The quantitative risk assessment model of Messens et al. (2007) suggests that reducing *Campylobacter* spp. levels by 1, 2 and 3 log CFU/ml could result in a reduction in the prevalence of *Campylobacter* spp. by 55%, 81% and 94% respectively. However, this model is not supported by the present study. Specifically, the enumeration of *Campylobacter* spp. in the experimental group decreased by 0.93 log CFU/ml as compared to the control group but the prevalence of *Campylobacter* spp. was only reduced by 1.34%. Therefore, this issue remains unresolved. Additional future studies may help bring a resolution here.

Finally, as a practical matter results from this study suggest that the administration of antibiotics via drinking water should be closely monitored. Specifically, results show that drinking water may be a prime source of bacterial cross contamination. Therefore, sound sanitation practices are critical in the control of *Campylobacter* contamination. To this end, water troughs should be regularly cleaned and disinfected with Nolvasan Solution.

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## CHAPTER 5 CONCLUSION

This dissertation research addresses several problems concerning the prevalence, enumeration, and control of *Campylobacter* spp. and *C. jejuni* in a small-scale poultry production setting. For the most part the results have successfully addressed these problems with new findings and insight. The most important conclusion from this research is that although the antibiotic sulfadimethoxine was shown to reduce the prevalence of *C. jejuni*, its application as an effective bacterial control is not supported.

This research also indicates that abiotic factors have the potential to contribute to horizontal cross contamination of *C. jejuni*. Most notably, water and feed represent likely sources for cross contamination. Therefore, sound sanitation practices are of paramount concern in small scale poultry farming. Although other sources have suggested that egg shells might provide a vertical means of cross contamination, this research found this not to be the case.

Because this research represents an initial inquiry in the control of *C. jejuni* in small scale poultry production, other question remain. It is contemplated that future research will provide additional evidence related to this issue. For example, future studies might explore sulfadimethoxine drug residue in chicken meat. This is a concern because antibiotic drug residue carryover may adversely affect the health of the consumer. Because *C. jejuni* is a bacterium, it has the potential to become resistant to antibiotics. Therefore, future study may address this issue with respect to the long-term use of sulfadimethoxine in poultry production. Additionally, future studies might explore the use of sulfadimethoxine to control other pathogenic bacteria that infect poultry. Among others these may include *E. coli* O157:H7 and *Salmonella typhimurium*.

In the present study, the presence of *C. jejuni* was detected via feces. However, other studies might contribute additional information by testing other matrices. As commonly practiced, these may include caeca, crop, and the intestinal tract. In the present study, biochemistry methods were employed. However, real-time PCR methods could be used to enhance the detection, isolation, and quantification of *C. jejuni*. PCR methods provide precise and timely results.

## **VITA**

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